THE ROLE OF PROSTAGLANDIN E2 IN CAUSING SUSCEPTIBILITY TOWARDS ANAPHYLAXIS

DISSERTATION

zur Erlangung des akademischen Grades

Doctor rerum naturalium
(Dr. rer. nat.)
im Fach Biologie

eingereicht an der
Lebenswissenschaftlichen Fakultät der Humboldt-Universität zu Berlin

von

M.Sc. Shruti Rastogi

Präsidentin der Humboldt-Universität zu Berlin

Prof. Dr.-Ing. Dr. Sabine Kunst

Dekan der Lebenswissenschaftlichen Fakultät
der Humboldt-Universität zu Berlin

Prof. Dr. Bernhard Grimm

Gutachter/innen

1. Prof. Dr. med. Margitta Worm
2. Prof. Dr. med. H. D. Volk
3. PD Dr. Nishith Gupta

Tag der mündlichen Prüfung: 09.07.2020

DIFFICULTIES IN YOUR LIFE DO NOT COME TO DESTROY YOU, BUT TO HELP YOU REALISE YOUR HIDDEN POTENTIAL AND POWER. LET DIFFICULTIES KNOW THAT YOU TOO ARE DIFFICULT.

– DR. A.P.J ABDUL KALAM (1931-2015)

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

15-PGDH-I	15 hydroxyprostaglandin dehydrogenase inhibitor
aIgE	Anti-immunglobulin E
β-ME	β-Mercaptoethanol
AERD	Aspirin exacerbated respiratory disease
AIA	Aspirin induced asthma
AIU	Aspirin induced urticaria
AKT	Protein kinase B
ANA	Anaphylaxis
ASA	Acetyl salicylic acid
APC	Allophycocyanin
ATA	Aspirin tolerant asthma
Bgl II	Restriction endonuclease from Bacillus globigii
BMcMCs	Bone marrow derived cultured mast cells
BSA	Bovine serum albumin
cAMP	Cyclic adenosine monophosphate
CCL	Chemokine ligand
cDNA	Complementary deoxyribonucleic acid
COX/s	Cyclooxygenase/s
CTMC	Connective tissue mast cells
cKIT	CD117/stem cell factor receptor
Cys-LT/s	Cys-leukotriene/s

Δ Tmax.	Maximal temperature difference
DAG	Diacylglycerol
DAPI	4′,6-diamidin-2-phenylindol
DMEM	Dulbecco’s modified eagle’s medium
DMSO	Dimethyl sulphoxide
DNA	Deoxyribonucleic acid
EAACI	European Academy of Allergy and Clinical Immunology
ECL	Enhanced chemiluminescence
ELISA	Enzyme-linked immunosorbent assay
EP receptors	E-prostanoid receptors
ERK	Extracellular signal-regulated kinase
FACS	Fluorescence-activated cell sorting
FcεRI	Fc-epsilon-receptor 1
FCS	Fetal calf serum
GAB2	GRB2-associated binding protein 2
GPCRs	G-protein-coupled receptors
GRB2	Growth factor receptor bound protein 2
HCl	Hydrochloric acid
HRP	Horseradish peroxidase
HuMC/s	Human mast cell/s
IB	Immunoblotting
Ig	Immunoglobulin
IgER	Immunoglobulin E receptor

IL	Interleukin
i.p.	Intra peritoneal
IP	Immunoprecipitation
IP3	Inositol triphosphate
ITAM	Immunoreceptor tyrosine-based activation motifs
i.v.	Intra venous
JNK	c-Jun N-terminal kinase
KCl	Potassium chloride
LAT	Linker for activation of T-Cells
LB	Lysogeny broth
LT/s	Leukotrienes
LTC4	Leukotriene C4
LTD4	Leukotriene D4
LTE4	Leukotriene E4
MACS	Magnetic activated cell sorting
MAPK/s	Mitogen activated protein kinase/s
MC/s	Mast cell/s
mrIL3	Mouse recombinant IL3
$MgCl_2$	Magnesium chloride
MMC/s	Mucosal mast cell/s
mMCP	Mouse mast cell protease
NaCl	Sodium chloride
Na_2CO_3	Sodium bicarbonate

NaOH	Sodium hydroxide
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B-cells
NFAT	Nuclear factor of activated T-cells
NSAID/s	Non-steroidal anti-inflammatory drug/s
NTAL	Non-T cell activation linker
OVA	Ovalbumin
p38	p38 MAP kinase
PAF	Platelet activating factor
PAG-CM	PIPES albumin glucose-calcium magnesium
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with tween-20
PE	Phycoerythrin
PI3K	Phosphoinositide 3-kinase
PIP2	Phosphatidylinositol-4,5-bisphosphate
PIP3	Phosphatidylinositol-3,4,5-trisphosphate
PIPES	1,4-piperazinediethanesulphonic acid
PGD_2	Prostaglandin D2
PGE_2	Prostaglandin E2
PGF_2	Prostaglandin F2
PGG_2	Prostaglandin G2
PGH_2	Prostaglandin H2
PGHS	Prostaglandin H synthase
PGI_2	Prostaglandin I2

PKA	Protein kinase A
PLA2	Phospholipase A2
PLCγ1/PLC γ2	Phospholipase C gamma 1/2
PKC	Protein kinase C
PMSF	Phenylmethylsulphonyl fluoride
PSA	Passive systemic anaphylaxis
PTGER	Gene name for prostaglandin E receptor
PVDF	Polyvinylidene fluoride
qPCR	Quantitative polymerase chain reaction
RPM	Rotations per minute
RT-PCR	Real time polymerase chain reaction
SH2	SRC homology 2
sIgE	Specific immunoglobulin E
SCF	Stem cell factor
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SLP76	SH2 domain containing leukocyte protein of 76kDa
t_{An}	Annealing time
t_{Den}	Denaturation time
t_{Elong}	Elongation time
TBP	TATA-binding protein
TBST	Tris buffered saline with tween-20
TEMED	Tetramethylethylenediamine
TH2	T helper 2

TNF-α	Tumor necrosis factor-alpha
TNP	Trinitrophenol
WAO	World Allergy Organization
Xba I	Restriction endonuclease from Xanthomonas badrii

1. ZUSAMMENFASSUNG

Die Ausbildung und der Schweregrad einer Anaphylaxie kann durch verschiedene Co-Faktoren beeinflusst werden. Zu diesen zählen die nichtsteroidalen Antiphlogistika (NSAIDs), die ihre Wirkung über die Inhibition der COX entfalten. Wie NSAIDs den Schweregrad der Anaphylaxie beeinflussen, ist bisher nicht genau bekannt. Interessanterweise zeigen Anaphylaxie-Patienten mit einer NSAID-Hypersensibilität niedrige Konzentrationen des regulatorischen Prostaglandins E2 (PGE_2). Zudem zeigen ASA-tolerante und –intolerante Asthma-Patienten variable anaphylaktische Sensitivitäten.

Anhand der vorliegenden Arbeit sollte untersucht werden, ob sich eine PGE_2-Dysregulation auf die Ausbildung und den Schweregrad der Anaphylaxie auswirkt und ob diese durch genetische Prädispositionen gefördert werden kann.

Dazu wurden zunächst die PGE_2 Konzentration im Serum von ANA-Patienten und gesunden Individuen gemessen. ANA-Patienten zeigten reduzierte PGE_2 Level, die invers mit dem Schweregrad der ANA korrelierten. Unterstützend weisen zwei in der Allergieforschung häufig verwendete Mauslinien, Balb/c und C57BL/6, unterschiedliche PGE_2 Level auf, die wiederum invers mit dem ANA-Schweregrad korrelierten. Eine Stabilisierung der PGE_2 Konzentration mittels eines pharmakologischen Inhibitors der Hydroxyprostaglandin-Dehydrogenase (15-PGDH-I) *in vivo* führte zu einer Verbesserung des ANA Schweregrades. Um in diesem Zusammenhang den Einfluss von ASA und PGE_2 besser zu verstehen, wurde das Model der systemisch passiven ANA im Mausmodel eingesetzt. ASA verschlimmerte den Schweregrad der ANA durch die Inhibition der COX1/2. PGE_2 konnte diese Verschlimmerung über die EP Rezeptoren 2, 3 und 4 reduzieren. Um die zugrundeliegenden Mechanismen der Wirkweise von exogenem PGE_2 und EP-Agonisten besser zu verstehen, wurden diese Zusammenhänge in murinen und humanen Mastzellen untersucht. PGE_2 reduzierte die Schwere der ANA durch Inhibition der Mastzell-Aktivität in diesem System über die Rezeptoren EP2 und EP4.

Anhand der vorliegenden Arbeit konnte gezeigt werden, dass bereits homöostatische PGE_2 Konzentrationen die Aktivität der Mastzelle verändern und vor einer schweren ANA schützen. Zudem kann der Grad der ANA und der Einfluss des PGE_2 auf die Mastzellantwort durch genetische Prädisposition beeinflusst werden. Die pharmakologische Stabilisierung des PGE_2 könnte daher eine vielversprechende, therapeutische wie auch vorbeugende Strategie zur Behandlung risikoreicher ANA-Patienten sein.

2. SUMMARY

The clinical outcome of anaphylaxis (ANA) can be affected by several co-factors. Non-steroidal anti-inflammatory drugs (NSAIDs) are well-known co-factors of ANA acting via COX-inhibition. The NSAIDs-mediated mechanisms altering the severity of ANA are not well-defined. It is reported that patients of ASA (NSAID)-hypersensitivity show low levels of the regulatory prostaglandin E2 (PGE_2). Moreover, the effectiveness of PGE_2 administration in such patients suggests a critical role of PGE_2 in ASA hypersensitivity. In addition, patients of ASA-tolerant and ASA-intolerant asthma show variable ANA sensitivities suggesting a role of genetic variation in susceptibility.

The aim of this thesis was to study whether and how PGE_2 dysregulation predisposes to ANA and whether genetic pre-dispositions affect the PGE_2 system and therefore ANA susceptibility.

First, sera from ANA patients and healthy individuals were analyzed for PGE_2 levels. ANA patients were characterized by reduced PGE_2 levels which inversely correlated with the severity of ANA. This disparity was confirmed by differential PGE_2 levels between Balb/c and BL/6 strains, two genetic mouse strains frequently employed in allergy research. PGE_2 levels in these mice were again inversely related with the severity of ANA. Results were confirmed by *in vivo* PGE_2 stabilization using 15-hydroxyprostaglandin dehydrogenase inhibitor (15-PGDH-I). Pharmacological PGE_2 stabilization ameliorated ANA severity in mice. A passive systemic ANA (PSA) model was applied to study the impact of ASA on ANA severity and the role of PGE_2 in this context. ASA aggravated ANA by inhibiting COX-1/COX-2, while PGE_2 reduced the aggravation through EP receptors 2, 3 and 4. To delineate the underlying mechanisms, murine and human mast cells were used to study the impact of exogenous PGE_2 and EP agonists. PGE_2 attenuated ANA severity by inhibiting MC activation through EP2 and EP4 receptors and interfering with MC signaling.

In summary, this thesis demonstrates that homeostatic levels of PGE_2 modulate MC activation and protect against ANA severity. The impact of PGE_2 on MC responses and ANA susceptibility is governed by genetic variation. Pharmacological stabilization of PGE_2 may prove to be a therapeutic or preventive strategy in the management of high-risk ANA patients.

3. INTRODUCTION

3.1 Type I Allergy

A type I allergic reaction is based on a mast cell (MC) and Immunoglobulin E (IgE)-dependent hypersensitivity towards foreign proteins known as allergens.[1] Allergic or hypersensitivity reactions are classified into four types (Type I-IV) by Coombs and Gell. [2] Allergy, the most common type of hypersensitivity is often equated with type I hypersensitivity reactions or immediate hypersensitivity mediated by IgE antibodies. It manifests in the form of urticaria, seasonal allergy, asthma, food allergy or anaphylaxis (ANA). [3] The latter one is the most severe manifestation[4] of an allergic reaction. When the allergen is first encountered, B-cells are stimulated by $CD4^+$ TH2 (T-helper 2) cells to produce IgE antibodies specific against the allergen.[5-9] During sensitization, specific IgE antibodies bind to IgE (FcεRI) receptors on the surface of tissue MCs and blood basophils. A subsequent exposure to similar allergen leads to the cross-linking of bound IgE on the surface of sensitized cells and results in the release of pro-inflammatory substances (section 1.2.2) which mediate the allergic symptoms.[1, 10] The prevalence of allergic diseases in most industrialized countries has increased dramatically in the past 50 years affecting 20-30% of the populations.[11, 12]

3.2 Mast Cells

MCs are cells of the hematopoietic lineage which originate from pluripotent precursor cells of the bone marrow. The discovery of IgE-mediated release of histamine from sensitized tissue MCs [13] led to the recognition of MCs as effector cells of Type I hypersensitivity. Antigen-dependent activation of tissue MCs bound to specific IgE on their surface and the release of biologically active mediators is the central event in acute allergic and anaphylactic reactions. [14, 15]

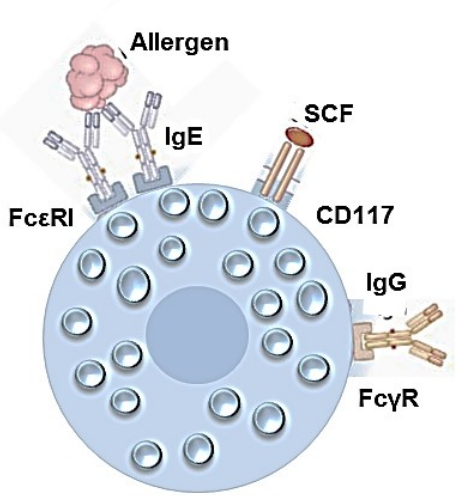

Figure 1: The Mast Cell. Mast cells are granulated cells with several receptors on their surface, the most important ones being FcεRI, CD117 and FcγR and their ligands IgE, SCF and IgG respectively are the major activators of mast cells. Mast cells can be activated by a multitude of stimuli and there are many modes of mast cell response. Responses to activation include the release of pre-formed mediators stored within granules and the synthesis and release of new products.

MCs arise from $CD13^{+}CD34^{+}CD117^{+}$ hematopoietic progenitors in the bone marrow [16], but mature locally, often residing near surfaces exposed to allergens. The major factors for MC growth and development include interleukin 3 (IL-3), stem-cell factor (ligand for the receptor tyrosine kinase Kit) and TH2-associated cytokines such as IL-4 and IL-9. MCs possess several receptors on their surface for different ligands most importantly CD117 for SCF and the high-affinity FcεRI, for IgE [17] (Fig.1). The manifestations of MC reactions are considered to be a consequence of the release of pro-inflammatory mediators following antigen-induced aggregation of IgE-bound FcεRI receptor.[10, 18-20]

MCs have a widespread tissue distribution and are predominantly found at the interface between the host and the external environment such as respiratory mucosa, skin and gastrointestinal tract.[17] They do not represent a homogenous population. Their differentiation and maturation is influenced by the microenvironment, resulting in morphological, biochemical and functional differences.[21] Rodent MCs subtypes can be divided based on their tissue distribution into two types: connective tissue mast cells (CTMCs) in the skin and peritoneal cavity and mucosal mast cells (MMCs) in the intestinal propria.[22-24] These subtypes of MCs vary in their size, histamine content, proteoglycan and neutral protease content which determine their staining characteristics.[25, 26] They show differences in function, including variable

responsiveness to different substances.[27] Human MCs (HuMCs) in the skin, lung and small intestine are classified based on the protease composition of their secretory granules into two types: connective tissue MCs (MC_{TC}) cells containing tryptase, cathepsin G and carboxypeptidase; mucosal MC (MC_T) containing only tryptase.[28, 29] Human and rodent MCs show a lot of heterogeneities like their dependence on growth factors and the contents of their secretory granules.[30]

3.2.1 Mast cell signaling pathway

MCs play a role in both innate and adaptive immune responses. [31, 32] Antigen-dependent MC activation takes place classically by cross-linking of the FcεRI receptor. [10, 33] FcεRI is a tetrameric receptor that comprises an α-chain, responsible for binding IgE, a β-chain and a disulphide linked γ-chain homodimer, responsible for initiating signaling. [34, 35] FcεRI-aggregation induces a cascade of intracellular signaling events (Fig. 2), which requires the translocation of FcεRI to lipid rafts within the plasma membrane. [36]

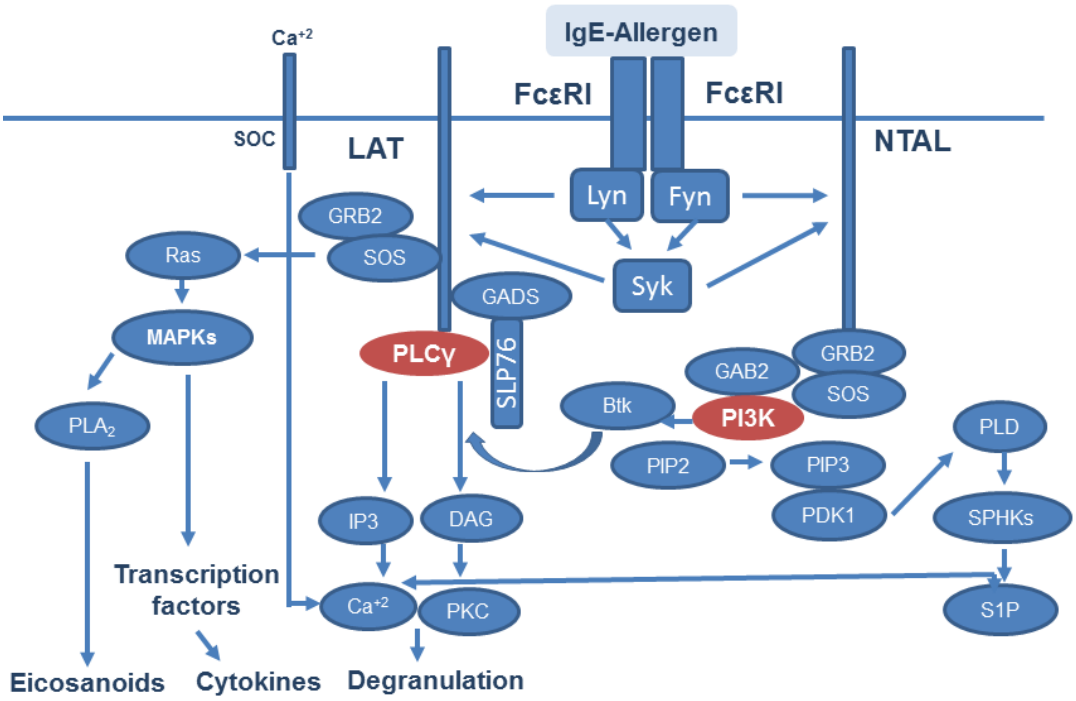

Figure 2: Mast Cell Signaling Pathway. The activation of MCs following the allergen mediated cross-linking of FcεRI receptor on MCs involves the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) through Lyn/Fyn followed by a cascade of tyrosine kinase phosphorylation of various proteins and the recruitment of different adaptor proteins. This cascade leads to the activation of phospholipase C (PLC) through this complex or through phosphoinositide 3-kinase (PI3K) which induces Ca^{+2} mobilization and degranulation. Cytokine production is regulated by several transcription factors. This illustration is based on the reference [37].

In a primary pathway, the signaling FcεRI β- and γ-chains are tyrosine phosphorylated by the action of the Src kinase Lyn, which is constitutively activated within the lipid rafts. [38] The phosphorylation sites within the β and γ chains contain immunoreceptor tyrosine-based activation motifs (ITAMS) which provide docking sites for the Src homology 2 (SH2) domains of associated signaling molecules.[39] The phosphorylated ITAMs of the γ-chains recruit the tyrosine kinase Syk, which phosphorylates downstream substrates after its phosphorylation including the adaptor transmembrane molecule linker for activation of T cells (LAT).[35, 40] The multiple tyrosines phosphorylated on LAT allow the recruitment of several signaling molecules like growth-factor-receptor-bound protein 2 (GRB2)-related adaptor protein (GADS), SH2-domain-containing leukocyte protein of 76 kDa (SLP76), VAV and the formation of a receptor-signaling complex [41]. This is followed by the activation of phospholipase Cγ1 (PLCγ1) and phospholipase Cγ2 (PLCγ2) [42] via the cytosolic adapter molecules GADS and SLP76.[43] PLC catalyzes the hydrolysis of phosphatidylinositol (4,5)- bis phosphate (PIP2) forming inositol (1,4,5)-triphosphate (IP3) and diacylglycerol (DAG) [44] which liberate Ca^{2+} from the endoplasmic reticulum and induce the activation of protein kinase C (PKC) respectively.[45] PLCγ activation occurs through this complex and through phosphoinositide 3-kinase (PI3K)-mediated membrane recruitment of bruton's tyrosine kinase (BTK) and phosphorylation of PLC by BTK. [46]

Another complementary pathway (Fig. 2), involves the activation of tyrosine kinase Fyn and Syk inducing a signaling complex with VAV, GRB2, GRB2-associated binding protein 2 (GAB2) and PI3K, organized by non T-cell activation linker (NTAL)/linker for activation of B cells (LAB). [47] PI3K catalyzes the phosphorylation of PIP2 to form PIP3.This molecular configuration allows these molecules to be recruited into the receptor-signaling complex. PI3K activation leads to the membrane recruitment of 3-phosphoinositide-dependent protein kinase 1 (PDK1), which activates PKCδ, ultimately leading to degranulation. Sphingosine kinases (SPHKs) can be activated by phospholipase D (PLD), Lyn or Fyn. SPHK activation regulates Ca^{+2} release and drives PKC and nuclear factor kappa-light-chain-enhancer of activated B-Cells (NFκB) activation, degranulation, eicosanoid production and cytokine synthesis. [48-51] GRB2 and son-of-sevenless homologue (SOS) are associated with both the primary and complementary pathways and activate RAS followed by extracellular-signal-related kinase (ERK) mitogen-activated protein kinase (MAPK) cascade and eicosanoid

production by activation of phospholipase A2 (PLA2). [52] Cytokine expression is regulated by various transcription factors [53] and by PI3K, PKC, Ca^{+2} and the downstream activation of other transcription factors such as NFκB and nuclear factor of activated T-Cells (NFAT). [53, 54]

3.2.2 Mast cell mediators

The activation of MCs results in the release of various pro-inflammatory mediators (Fig. 3) which fall into three categories:

1) Pre-formed mediators [21] like histamine, β-hexosaminidase (β-hex), serotonin (mainly produced by MCs from mouse and rat) [55] or proteases (tryptase, chymase), stored in the cytoplasmic granules.

2) De novo produced lipid mediators[21] like Cys-leukotrienes (Cys-LTs) and prostaglandins (PGs), which are formed from arachidonic acid. PGD_2 is released in large quantities from MCs, while the leukotriene predominantly produced is LTC4 (Leukotriene C4), which is metabolized to LTD4 (Leukotriene D4), and LTE4 (Leukotriene E4). [56]

3) Cytokines, chemokines and growth factors. [21]

While the first two classes of mediators are released within minutes of antigen contact, cytokines and chemokines are released within 1-4 h of MC activation.[17, 56, 57] The inflammatory factors increase vascular permeability leading to vasodilation, angioedema and urticaria. [10, 58, 59] In addition, they can cause bronchoconstriction and hypotension, leading to anaphylactic shock in the worst case. [10, 58, 59]

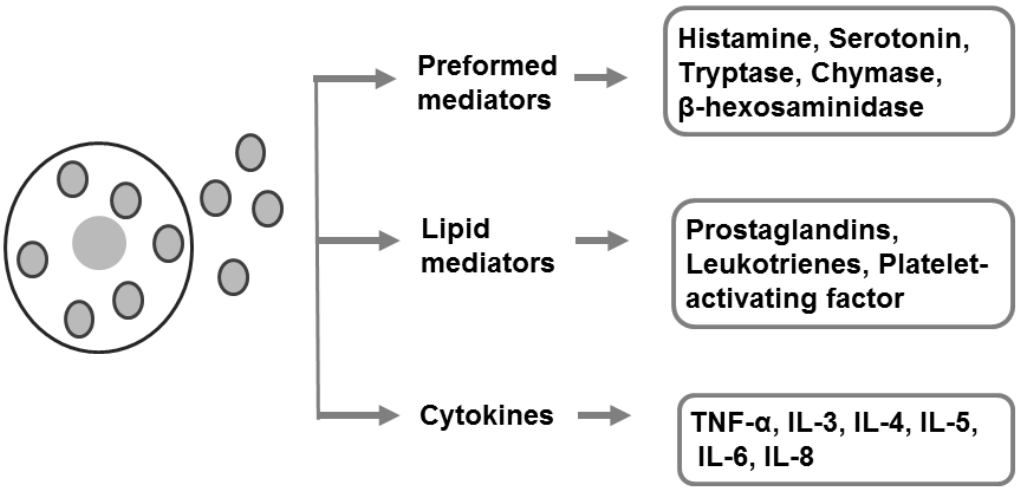

Figure 3: Murine and human MC (HuMC) mediators. Shown are the major MC mediators released on IgE-mediated cell activation. This illustration is based on the references [56, 57, 60]. TNF -α, tumor necrosis factor; IL, interleukin.

MCs differ based on species and their location in the body. [57] Their mediator profile also varies. For example the pre-formed mediator serotonin is produced in large quantities by murine MCs whereas HuMCs produce serotonin only in small concentrations.[55] There are also differences regarding tryptase and chymase between MCs from both species. Murine MCs produce a number of tryptases and chymases (mMCP-1 to mMCP-14) [57], whereas HuMCs produce only four tryptases (α-,β-,γ-,δ-tryptases) and one chymase (α-chymase). [61] Human and murine MCs also vary regarding their secretion of cytokines like IL-4, which is formed only by murine MCs under physiological conditions. [56, 57, 62]

3.3 Anaphylaxis

ANA is an acute, potentially life-threatening, systemic hypersensitivity reaction, characterized by the release of mediators from MCs and other inflammatory cells which lead to a rapid onset of respiratory, cutaneous, gastrointestinal and cardiovascular symptoms. [63-65]

The term anaphylaxis was originally coined by Charles Richet and Paul Portier in 1902. In their experiments with immunized dogs with actinia extracts [66] they observed the sudden onset of death instead of expected prophylaxis generating the term ‘anaphylaxis’. For this discovery Richet was awarded with the Nobel Prize in 1913. An

important breakthrough to the understanding of the pathophysiology of ANA was the data from Dale and Laidlaw. They demonstrated that histamine induced similar symptoms to ANA. [67] In the following years, it was observed that similar symptoms can be elicited in animals without the involvement of the immune system as well as through the direct injection of histamine. Such reactions were termed as 'anaphylactoid reactions'. [68] At the beginning of the 21st century, the nomenclature task forces of the European Academy of Allergy and Clinical Immunology (EAACI) and the World Allergy Organization (WAO), defined 'anaphylaxis' on the basis of clinical symptoms independent of the pathomechanism causing the reaction.[65] ANA describes a syndrome of clinical symptoms involving several organ systems with variable intensities. There are different severity systems used to classify the intensity of this reaction as proposed by Mueller[69] or Ring and Messmer [70] which are most commonly used.

3.3.1 IgE-dependent anaphylaxis

IgE antibodies play a significant role in conferring immunogenic specificity to effector cell activation in allergic reactions (section 1.1).[10, 14, 71] IgE is often increased in large quantities in patients suffering from allergic diseases [10, 72]. The effector cells of ANA, MCs, blood basophils, neutrophils, eosinophils, monocytes, dendritic cells and platelets express the high affinity receptor for IgE, FcεRI, on their surface.[73] Classically, ANA is induced when cells are exposed to a bi- or multi-valent allergen, by crosslinking of the FcεRI receptor-bound IgE. Crosslinking of IgE and its receptor induces a signaling cascade (Fig. 2) that induces activation of MCs or basophils. This results in the release of pre-formed mediators such as histamine, serotonin, cytokines, proteases, along with *de novo* synthesis of additional cytokines and lipid mediators such as platelet-activating factor (PAF), PGs, and LTs. [10, 73] Passive immunization studies including naive mice sensitized by transfer of antigen-specific IgE followed by exposure to that antigen, support the importance of IgE and MCs in antigen-induced anaphylactic shock. [74-76] In both passive and active immunization studies, the IgE-mediated anaphylactic reactions are completely abolished in mice with genetic or antibody elimination of IgE, the FcεRI receptor [75], or MCs [77-79], pointing to the importance of IgE-mediated MC activation in these ANA models.

3.3.2 IgE-independent anaphylaxis

In mice, IgG antibodies are also known to induce passive systemic anaphylaxis (PSA) similar to an IgE-dependent reaction. Studies in which mice were actively immunized with an antigen followed by exposure to the same antigen, have demonstrated ANA in the absence of classical IgE/FcεRI/MC pathway. These reactions were mediated by mechanisms involving IgG instead of IgE. [58, 80-83] The existence of IgE-independent ANA in actively immunized mice was clearly demonstrated by studies in IgE- or FcγRIα-deficient mice but not in mice lacking stimulatory FcRγ receptors. Suppression or lack of ANA in mice was seen in mice lacking function of one or more mouse FcRγ receptors. [84-87]

Studies of murine IgG-mediated ANA in actively immunized mice identified PAF rather than histamine as the most important mediator and monocytes/macrophages, basophils or neutrophils as the critical cell types.[81, 82, 84] These cell types express FcRγIII and FcγRIV in mice, and are capable of producing PAF in response to appropriate stimuli.[82, 88, 89] Several clinical observations support the importance of IgG-mediated ANA in human subjects [90-93] particularly in the presence of relatively high titers of specific IgG antibody and large quantities of known antigen. As demonstrated in mice, IgG-mediated ANA requires a larger dose of antigen than IgE-mediated ANA. [94]

3.3.3 Complement-mediated anaphylaxis

Studies using mice have shown that small peptides, C3a and C5a, derived from C3 and C5 respectively, known as anaphylatoxins**,** can activate MCs and other myeloid cells. [95] An activation of the complement cascade occurs in response to many stimuli, and leads to generation of small peptides, which are potent inflammatory mediators. [96] There is plenty of evidence indicating that anaphylatoxins might be involved in ANA. The production of C3a and C5a has been reported in human ANA. [97, 98] The injection of low doses of small peptides into the skin of healthy subjects led to wheal and flare reactions. [99-102] In addition, another study has shown the correlation between blood levels of small peptides and the severity of ANA in humans. [98] Several transgenic mice models suggest that the effect of complement components on ANA may be largely redundant and depend on the specific model used. [103-105]

3.3.4 Co-factors of anaphylaxis

The mechanisms involved in anaphylactic reactions are complex and implicate various pathways. Some of these mechanisms may be key to the development of a reaction, while others may only modify its severity. Clinical manifestations of ANA are heterogeneous with signs and symptoms varying across patients. Presentation and severity can be affected by factors that are independent of the triggering allergen but modulate occurrence, severity and the course of a reaction. This has led to the concept of co- or augmenting factors. Around 40% of severe anaphylactic reactions have been reported to be influenced by such extrinsic factors. [106-109] Studies suggest two major mechanisms of cofactor-induced modulation of ANA: an increased bioavailability of the allergen and a decreased activation threshold on the cellular level (Fig. 4).[106] Exercise, vulnerability to psychological burden, and drugs like non-steroidal anti-inflammatory drugs (NSAIDs) are major extrinsic factors. Intrinsic factors such as higher age, male sex and concomitant mastocytosis may also facilitate a reaction or aggravate its outcome. [106-108] Other factors like alcohol, infectious diseases and estrogens are also considered as important co-factors. [108, 110, 111]

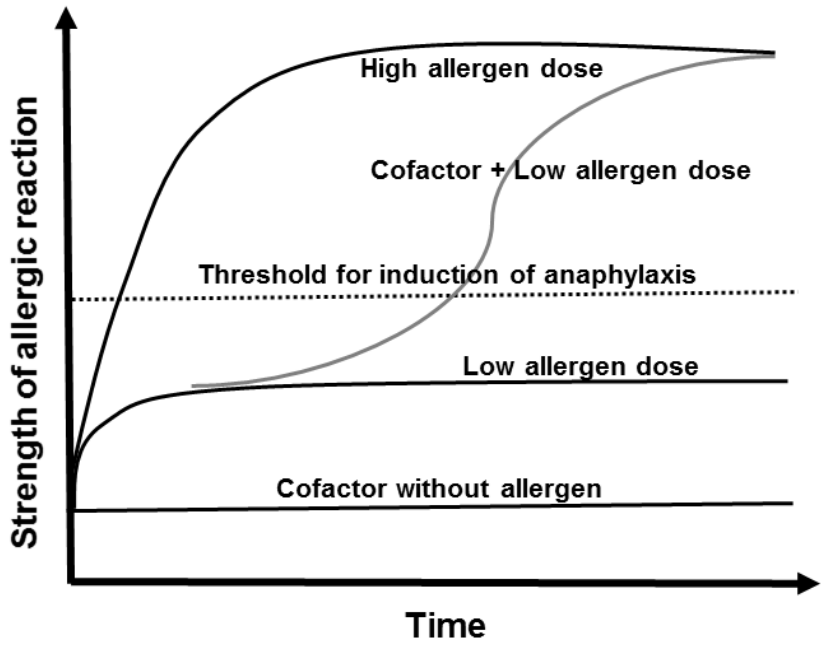

Figure 4: 'Threshold dose' model of cofactor-dependent anaphylaxis. High allergen doses induce strong ANA, while low allergen doses induce subclinical allergic reaction but no ANA. In contrast, low 'subthreshold' allergen doses in combination with cofactors trigger strong ANA. This illustration is based on the reference [106].

3.3.5 NSAIDs as co-factors of anaphylaxis

NSAIDs are a well-known group of augmentation factors of ANA. The first report of a life threatening hypersensitivity reaction upon ingestion of peanuts in combination with aspirin was published in 1984. [112] Epidemiological data suggests that NSAIDs act as a trigger in 1.2-4.7% of all reported anaphylactic cases. [106] They have been reported to be present in up to 22% of cases of food-induced severe ANA. [113] Other reports have shown NSAIDs in 58% of co-factor induced food-related ANA reactions in the Mediterranean area [114] and in around 33% of cases in lipid transfer protein (LTP)-induced ANA. [115]

Studies have shown that the augmenting effect of NSAIDs in food allergic reactions may be related to the blockade of COX pathway and the decreased synthesis of PGs.[116-119] It is very well known that PGs play an important role in maintaining homeostasis [120, 121] (section 1.4) including the defense and repair of gastrointestinal tissues. NSAIDs inhibit PGs, disposing gastrointestinal tissues to injury and less capacity to retrieve mucosa function.[122] NSAIDs especially acetyl salicylic acid (ASA) is also known to cause hypersensitivity reactions known as NSAID-exacerbated respiratory disease (NERD) or ASA-induced asthma (AIA). These hypersensitivities are characterized by respiratory symptoms such as bronchospasms, acute asthma exacerbation (lower airway), and severe asthma morbidity. [123, 124]

3.4 Cyclooxygenases and Prostaglandins

Upon activation, MCs release arachidonic acid from cell membrane phospholipids by the action of PLA2 and PLC (Fig. 5). [125] Arachidonic acid is in turn converted into Prostaglandin G2 (PGG_2) and Prostaglandin H2 (PGH_2) by the action of an enzyme called prostaglandin endoperoxide synthase, fatty acid cyclooxygenase, prostaglandin H synthase (PGHS) or most commonly, cyclooxygenase or COX.[126] There are two isoforms of COX- , a constitutive COX-1 and an inducible COX-2, which differ in their regulation of expression and tissue distribution. COX-1 is responsible for the physiological production of PGs in the stomach, kidney, ovaries and platelets, whereas COX-2 is the inducible enzyme responsible for the pathological production of PGs in various diseases.[127] Further enzymatic conversion of PGH_2 by terminal prostanoid

synthases [126] and various isomerases leads to the formation of PGE_2, PGD_2, PGI_2, PGF_{2a} and the related thromboxane A_2. [128]

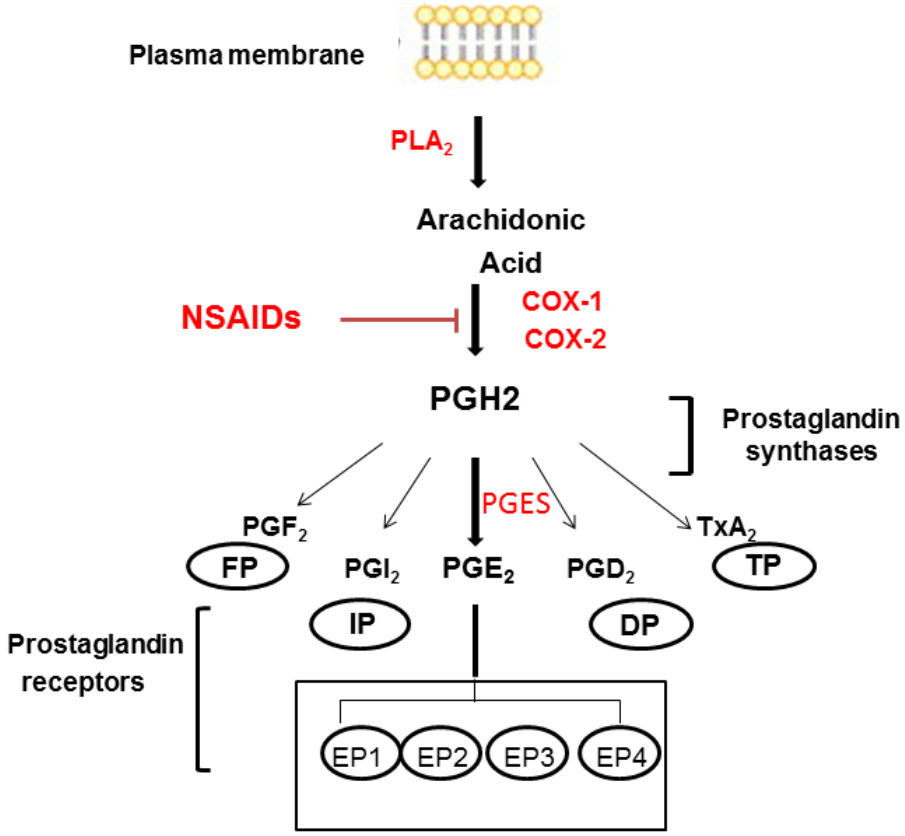

Figure 5: Prostaglandins synthesis and receptors. Arachidonic acid is released from the membrane phospholipids and converted to prostaglandin H2 (PGH_2) by cyclooxygenases-1/2 (COX-1 and COX-2). COX activity is inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) therefore reducing the synthesis of prostaglandins. Prostaglandins act via their specific receptors. This illustration is based on the reference [129].

Prostaglandins are 20-carbon fatty acid derivatives of mainly arachidonic acid with important roles in homeostasis and inflammation. They are produced by almost every cell of the body.[120, 130, 131] They are not stored by cells rather synthesized either constitutively or in response to cell-specific trauma, stimuli or signaling molecules. [131] PGs mediate pain and fever [132], and COX-inhibitors i.e. NSAIDs are the most commonly used anti-inflammatory, and anti-pyretic drugs. They act by inhibiting the activity of COX enzymes (COX-1 and COX-2) and the consequent reduction in prostaglandin levels.[133]

As mentioned earlier, NSAIDs/ASA represent a prominent group among the co-factors of ANA. [4, 106] A large body of evidence supports a close link between the inhibition of COX and the manifestation of an allergy/hypersensitivity (table 1).

Model used	Results	Publication
Allergic airway disease mouse model (COX deficient/ WT mice)	COX protects from lung inflammation after allergen challenge. COX-1 has greater effect than COX-2.	Gavett et al. 1999 [134]
Allergic airway disease mouse model	COX inhibitors increase AHR. COX-1 is dominant over COX-2.	Peebles et. al 2000 [135]
Allergic airway disease mouse model (COX-1/-2 deficient/WT mice)	COX-2 reduces bronchoconstriction	Carey et. al 2003 [136]
Allergic airway disease mouse model (COX-2 deficient/WT mice)	Reduced PGE_2 associated with COX-2 deficiency	Nakata et. al 2005 [137]
Allergic pleurisy mouse model	PGE_2 downregulates allergic response and mediates subsequent hypo responsiveness	Bandeira-Melo et. al 1996 [138]

Table 1: Decreased COX activity associated with increased allergic symptoms. Reports showing the importance of COX in the manifestation of allergy/hypersensitivity. COX, cyclooxygenase; PGE_2, Prostaglandin E2.

3.5 Prostaglandin E2

PGE_2 is one of the most abundant PG subtypes produced by almost all cells and tissues throughout the body with a complex mode of action and versatile biological activities. [139-141] Under physiological conditions, PGE_2 is an important mediator of several biological functions such as the regulation of immune responses, blood pressure, gastrointestinal integrity, kidney function, skin homeostasis and fertility. [121, 130, 131, 142] PGE_2 has a wide range of (sometimes opposing) biological effects both in physiology and pathology. [130, 140, 143] Knockout studies in mice show that PGE_2 can exert both pro-inflammatory and anti-inflammatory responses. [144-147] Diversity and complexity related to PGE_2 is created at different levels: 1) PGE_2 synthesis, there are three specific PGE_2 synthases (PGES)- cytosolic PGES (cPGES; constitutively expressed) and microsomal PGES (mPGES1/2;inducibly expressed). [126] 2) The existence of four PGE_2 receptors, named E-prostanoid/s (EP/s) 1 to 4 (Fig. 6).

The PGE_2 receptors are members of the G-protein coupled receptor (GPCR) family with distinct downstream effectors.[130] EP_1 receptors are coupled to Gαq/p and mediate signaling by activation of PLC which ultimately results in the increase of intracellular Ca^{2+}.[140] EP_2 and EP_4 receptors are coupled to the stimulatory Gαs and mediate the stimulation of cAMP/PKA signaling. [148] The EP3 receptor is unique and has different isoforms (3 in mice and 12 in humans), which are coupled to either the inhibitory Gi/G_q or Gs proteins thereby mediating different effects. [149, 150] Diverse factors regulate the outcome of EP receptor signaling. The structural, pharmacological and functional differences between the receptors influence the biological effects of PGE_2. [143] More importantly, the characteristic pattern of expression as well as their relative ratios in different cells/tissues can cause diversity in the functions of PGE_2. [151-154] Despite the pro-inflammatory functions of PGE_2, it has a protective function in allergic diseases. [155, 156]

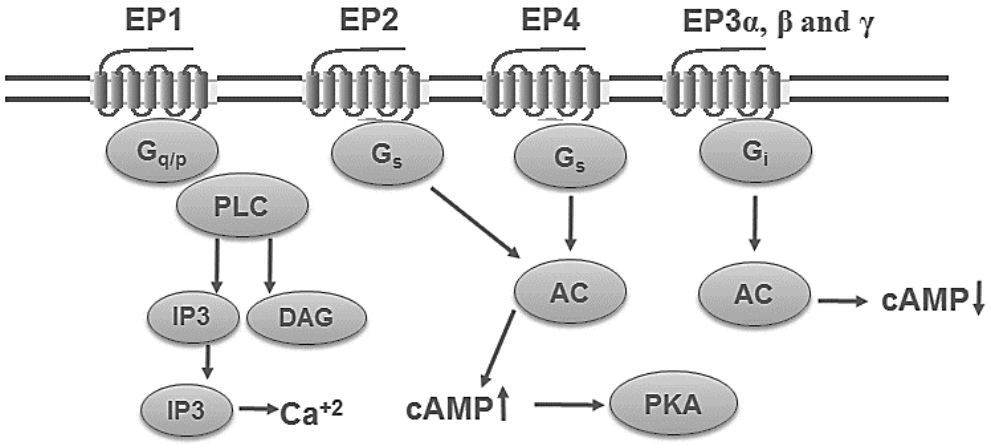

Figure 6: PGE_2 signaling pathways [145]**:** PGE_2 acts via four G-protein coupled receptors: E-prostaglandin (EP) 1-4. EP1 receptor is Gq-coupled and its activation increases Ca^{+2} mobilization through Ca^{+2} channels via phospholipase C (PLC). EP3 receptor is Gi-coupled and increases intracellular Ca^{+2} through PLC activation and/or blocks cAMP production via adenylate cyclase (AC). EP2 and EP4 receptors are Gs coupled and stimulate cAMP production via AC.

3.5.1 Prostaglandin E2 and Anaphylaxis

PGE_2 was originally regarded in the immune system as a contributor to the progression of inflammatory diseases. [131] However, the discovery of its bronchodilator property associated with asthma, first revealed its anti-inflammatory role. [157] Picado *et al.* [158] and Szczeklic *et al.* [159] first identified that nasal polyps and fibroblasts obtained from asthmatic patients express less COX-2/PGE_2 than airway cells from healthy donors. These reports proposed that the lack of PGE_2 production in asthmatics may partially

explain the onset of symptoms. These observations formed the pioneer attempts to better understand the protective role of PGE_2.

The well-known capacity of NSAIDs/COX-inhibitors to trigger asthma [160], and the occurrence of ASA hypersensitivity phenomena [161-163] provide major evidence for the protective role of COX/PGE_2 in the elicitation of allergic symptoms. [135, 164-166] The underlying causes are uncertain but reports suggest that inhibition of the COX pathway by NSAIDs/ASA leads to an increase in the metabolites of the lipoxygenase pathway. [162, 167] However, this theory has been contradicted by others. [168, 169] It is believed that lack of PGE_2 is responsible for the precipitation of allergic symptoms both in the lung and the skin. Studies have reported low levels of PGE_2 in patients with AIA compared to patients with ASA tolerant asthma (ATA) or healthy subjects. [170, 171] In addition, impaired production of PGE_2 and low COX expression has been reported in cells from patients of ASA-exacerbated respiratory disease (AERD). [159, 170, 172] Reduction of PGE_2, therefore, is termed as a characteristic of ASA-induced hypersensitivity. Another evidence comes from reduced EP2 expression levels in AIA [173] and a genetic variant of the EP4 gene (resulting in reduced expression) in connection with AIU. [174] The significance of PGE_2 as a critical component in ASA hypersensitivity is also supported by its (and synthetic analogues) effectiveness in ASA-induced bronchoconstriction. [175-177]

Strong evidence that PGE_2 may inhibit allergic inflammation is provided by animal studies using pharmacological and genetic methods to limit prostaglandin synthesis in a model of ovalbumin (OVA) induced lung allergy [135, 178]. Mice lacking EP3 receptors were reported to have increased allergic inflammation suggesting that a loss of PGE_2 is at least partly responsible for the enhanced inflammation in COX-deficient and NSAID treated animals. [166] PGE_2 has also been shown to limit inflammation in animal models of asthma. [179-181]

3.5.2 Prostaglandin E2 and Mast Cells

MC activation is a key event in the allergic inflammatory response. The expression of PGE_2 receptors by MCs [182, 183], combined with the close proximity to PGE_2-secreting cells, such as fibroblasts [184] and macrophages [185], suggest MCs as potential targets for immunoregulation by PGE_2. [153] Reports suggest that one possible explanation for

PGE_2's range of functions in allergies and asthma is its direct interaction with the different EP receptors on the surface of MCs. [151-154] The evidence comes from the involvement of MCs in the regulation of asthma [10], the ability of PGE_2 to limit MC degranulation [186] and the differential expression of PGE_2 receptors on subsets of human and murine MCs. [187]

MCs are known to show a wide range of responses towards PGE_2, but the literature does not homogenously point in one direction. PGE_2 has been reported to show potentiation of MC release in immunologically stimulated bone-marrow-derived MCs (BMMCs), peritoneal- derived MCs, human LAD2 cells and some cord-blood derived HuMCs including histamine/β-Hex, IL-6 and GM-CSF production. [153, 187-190] On the contrary, there are also reports including the same and other MC systems suggesting that PGE_2 may exert a dampening effect, *e.g.*: PGE_2-treated HuMC derived from cord blood [152], from blood progenitors [187, 190] or human lung MCs [154], display attenuated release of TNF-α, histamine/ β-Hex and Cys-LTs. Additionally, diminished releasability has also been shown by C57 MC line treated with PGE_2. [187]

This apparent dichotomy has been related to the multiple EP receptors on the surface of MCs. [151-154] MCs from different sites such as lung, peritoneum or blood-derived [151, 152, 154], different species [151-153] or different human donors [189] express varied levels of EP receptors and have shown different responses towards PGE_2. The comparison of different human and murine MCs, revealed that EP2 and EP3 receptors have dominant expression whereas EP4 is lowly expressed. [187] The expression of EP1 is virtually null. Only when EP2 is scarcely expressed in a given population, EP4 might be of biological importance.[191] A study by Serra-Pages *et al.* showed that the relative expression of EP2:EP3 is central in determining the cell response towards PGE_2. [187] However, it contrasted with another study suggesting that the heterogeneity of responses to PGE_2 result from differential coupling of EP receptors to signaling components rather than different EP2/3 ratios. [189] EP2 receptor has been shown to inhibit lung HuMC degranulation [151, 154], TNF and eicosanoid production in cord-blood derived HuMCs [152], Fyn-mediated signaling and hence FcεRI-mediated degranulation in PDMCs. [187] In contrast, the EP3 receptor can enhance antigen-mediated human and murine MC responses. [192, 193] The importance of EP4 receptor for transducing inhibitory actions of PGE_2 on MCs came into light only recently. [191] Overall, the literature shows a lot of paradox around the impact of PGE_2 on MCs.

4. AIMS AND OBJECTIVES

Prostaglandin E2 is a multifunctional prostanoid with an important role in immune regulation. A number of studies have suggested a protective role of COX and PGE_2 in the precipitation of allergic symptoms. Accordingly, reduced production of PGE_2 was found to be a characteristic of ASA (COX-inhibitor)-induced hypersensitivity. The aim of the present thesis was to study the PGE_2 system in association with ANA susceptibility and aggravation, and to delineate the underlying mechanisms.

The objectives of the study were:

1. To study the human PGE_2 system in ANA patients by comparison with healthy subjects.
2. To characterize the murine PGE_2 system by comparing two mouse strains frequently employed in allergy research for their susceptibility towards ANA and relation to PGE_2 deregulation.
3. To study the impact of ASA/COX-inhibitors on ANA severity and the role of PGE_2 using an *in vivo* model of ANA.
4. To pin down the biochemical events by which the PGE_2 pathway impacts MC responses *in vivo* and *in vitro* and to identify the molecular partners especially EP subset (s) involved.

5. MATERIALS AND METHODS

5.1 Materials

5.1.1 Cell lines

Cell line	Provider
HEK293	Cell Lines Service, GmbH, D

5.1.2 DNA clones and vectors

Clone/Vector	Provider
pTCN empty vector	transOMIC technologies, inc., USA
pTCN-PTGER2 (Homo sapiens)	transOMIC technologies, inc., USA
pTCN-PTGER3 (Homo sapiens)	transOMIC technologies, inc., USA
pTCN-PTGER4 (Homo sapiens)	transOMIC technologies, inc., USA

5.1.3 Reagents and Chemicals

Reagent/Chemical	Manufacturer
3,3',5,5'-Tetramethylbenzidin (TMB)	Sigma Aldrich, D
15-PGDH inhibitor, SW033291	Cayman Chemicals, D
100 bp DNA ladder	New England Biolabs GmbH, D
α-Monothioglycerol (α-MTG)	Sigma Aldrich, D
β-Mercaptoethanol	Sigma Aldrich, D
Acrylamide	Carl Roth, D
Ammonium per sulphate	Sigma Aldrich, D
Acetylsalicylic acid	Sigma Aldrich, D

Reagent/Chemical	Manufacturer
Agarose	Biozym, D
Aqua sterile	Braun, D
Bromophenol blue	Merck Millipore, D
Bgl II	New England Biolabs GmbH, D
Bovine serum albumin	PAA, A
Butanol	Merck Millipore, D
Carbenicillin	Carl Roth, D
Celecoxib	Cayman Chemicals, D
cKit magnetic beads	Miltenyi Biotec, D
Collagenase type I	Worthington, USA
Dispase	BD Biosciences, D
DMSO	Sigma Aldrich, D
DAPI	Sigma Aldrich, D
DNAase	Macherey-Nagel, D
ECL reagent westar ηC ultra 2.0	Cyanagen/ 7Bioscience GmbH, D
EDTA	PAA, A
Ethanol	J. T. Baker, D
Fugene-6	Promega GmbH, D
FCS	Biochrome, D
G418	Carl Roth, D
Glycine	Merck,D
Glycerol	Merck,D
Glucose	Sigma Aldrich,D
Heptan	Merck,D
Histamine	Sigma Aldrich,D
Human recombinant IL-3	Peprotech, D
Human recombinant SCF	Peprotech, D
Ionophore, A23187	Calbiochem, USA
Isoflurane	Abott Laboratories, USA
KCl	Sigma Aldrich, D
LB Agar	Sigma Aldrich, D

Reagent/Chemical	Manufacturer
LB Broth	Sigma Aldrich, D
L-glutamine	Biochrome, D
$MgCl_2$	Merck, D
Methanol	Merck, D
Milk powder	Carl Roth, D
Mouse recombinant IL-3	Immunotools, D
NaCl	Braun
Na_2CO_3	Merck, D
NaOH	Carl Roth, D
Non-essential amino acids	Biochrome, D
ONO-AE1-259-01 EP2	ONO Pharmaceutical Cp.,JP
ONO-AE-248 EP3	ONO Pharmaceutical Cp.,JP
ONO-AE1-329 EP4	ONO Pharmaceutical Cp.,JP
O-Pthalaldehyde	Sigma Aldrich, D
PGE_2	Cayman Chemicals, D
Phosphostop phosphatase inhibitor	Roche, D
PIPES	Sigma Aldrich, D
PMSF	Sigma Aldrich, D
Ponceau S	Serva Elektrophoresis, D
Protease inhibitor	Sigma Aldrich, D
Protein standard	Biorad, D
Penicillin/Streptomycin	Biochrome, D
PVDF membrane	GE Healthcare, USA
SC560	Cayman Chemicals, D
SDS	Sigma Aldrich, D
TAE-buffer (50X)	Genaxxon, D
TEMED	Sigma Aldrich, D
TNP-BSA	Igc, biosearch technologies, D
Trypan blue	Merck, D
Toluidine blue	Merck, D
Trizma base	Sigma Aldrich, D

Reagent/Chemical	Manufacturer
Triton-X	Merck, D
Tween-20	Sigma Aldrich, D
Xba I	New England Biolabs, GmbH, D

5.1.4 Antibodies

Antibody	Dilution Factor	Manufacturer
Anti-human IgE	1:500	KPL, USA
Anti-mouse IgE	1:500	BD Pharmingen, USA
Anti-TNP IgE	various	BD Pharmingen, USA
Anti-PTGER2 (H-75)	1:500	Santa Cruz Biotechnology, INC., USA
Anti-PTGER3 (H-200)	1:500	Santa Cruz Biotechnology, INC., USA
Anti-PTGER4 (C-4)	1:500	Santa Cruz Biotechnology, INC., USA
Anti-PTGER2	1:1000	Cayman Chemicals, D
Anti-PTGER3	1:200	Cayman Chemicals, D
Anti-PTGER4	1:200	Cayman Chemicals, D
Anti-PTGER3	1:200/1:500	Atlas Antibodies/Biozol GmbH, D
Anti-PTGER4	1:200/1:500	Atlas Antibodies/Biozol GmbH, D
cKit-APC	1:50	eBioscience, Thermo fischer, USA
Fcγ-Block	1:500	eBioscience, Thermo fischer, USA
FcεRI-PE	1:50	eBioscience, Thermo fischer, USA

Antibody	Dilution Factor	Manufacturer
Goat-anti-rabbit-HRP-labelled	1:10000	Chemicon International, Thermo fischer, USA
Human IgE	1:200	Calbiochem, USA
Rabbit Cyclophilin	1:1000	Cell Signaling Technology, D
Rabbit Phospho-AKT	1:250	Cell Signaling Technology, D
Rabbit Total-AKT	1:250	Cell Signaling Technology, D
Rabbit Phospho-ERK	1:1000	Cell Signaling Technology, D
Rabbit Total-ERK	1:1000	Cell Signaling Technology, D
Rabbit Phospho-P38	1:1000	Cell Signaling Technology, D
Rabbit Total-P38	1:1000	Cell Signaling Technology, D
Rabbit Phospho-PLCγ1	1:1000	Cell Signaling Technology, D
Rabbit Total-PLCγ1	1:1000	Cell Signaling Technology, D
TNP-IgE	1:500	BD Pharmingen, USA

5.1.5 Consumables

Material	Type	Manufacturer
Biosphere filter tips	0.5-20 µl	Sarstedt, D
	2-100 µl	
	100-1000 µl	

Material	Type	Manufacturer
Canula, sterile	21 G, 25G, 26G, 27G, 30G	BD, Pharmingen
Casy tubes		F. Hoffmann – La Roche
Cell culture flasks	25 cm_2, 75 cm_2, 175 cm_2	Sarstedt, D/ Thermo Fisher Scientific, D
Cell culture pates, sterile	12,48,96 wells	Nunc, D
Combi tips plus	0.5 ml, 5 ml, 10 ml, 12.5 ml	Eppendorf, D
Descosept AF		Dr. Schumacher GmbH, D
Eppendorf tubes	0.5 ml, 1.5 ml, 2 ml	BD Pharmingen, D
Eppendorf tube stand		TPP
FACS tubes		Sarstedt, D
Falcon	15 ml, 50 ml	BD, Pharmingen
Gloves sterile nitrile		Braun
RT tubes		Qiagen
Petri dishes		Greiner Bio-one, D
Pipette tips, sterile		Eppendorf
Pipetboy		TPP
Pipettes	(0.5-10 µl; 10-100 µl; 100-1000 µl)	Eppendorf
PVDF membrane (Amersham hybond-P)		Pharmacia Biotech
Serological pipettes	5 ml, 10 ml, 25 ml	Falcon, Thermo fisher Scientific
Scalpel	No.20	Feather, D
Syringes	10 ml, 20 ml, 50 ml	BD Pharmingen, D
Syringe filter		Sarstedt, D

Trans blot filter paper		Biorad Laboratories, D

5.1.6 Instruments

Technical Instrument	**Model**	**Company**
Agarose gel equipment		Biorad Laboratories, D
Autoanalyzer II		Borgwald Technik, D
Autoclave		Melag, D
Auto-MACS		Miltenyi Bitec, D
CASY technology cell counter		TTC Schärfe System GmbH
Chemiluminiscent detector	Fusion FX7 Spectra	Vilber Lourmet, D
Centrifuge	Varifuge RF	Heraeus,D
	Megafuge 1.0R	Heraeus D
Electrophoresis chamber		BioRad Mini Protean system
FACS	MACSQuant Analyzer	Miltenyi Biotec, D
Gradient thermocycler	Px2 Therma Cycler	Thermo Technologies, D
Incubator	HERA cell	Heraeus D
Light microscope	Axiovert 10	Carl Zeiss AG, D
Magnetic stirrer	Magnetmix 2070	Hecht-Assistant, D
Multichannel pipette		Eppendorf
Nanodrop spectrophotometer	1000	Thermo Fisher Scientific, US
pH-meter	pH 1100L	VWR International GmbH, D
Pipettes	10 µl, 20µl, 100 µl, 200 µl, 1000µl	Eppendorf, D

Technical Instrument	Model	Company
Power pack HC		Biorad, D
Rotorgene Q thermocycler	R1112155	Qiagen GmbH, D
Table centrifuge	5417 C	Eppendorf, D
	5417 R	
Thermometer Theralert	TH-5	Physitemp Instruments, LLC. USA
Water bath	3	Julabo, D
Victor reader multimode Plate Reader	X3	Perkin Elmer, D
Vortexer	Reax 2000	Heidolph, D

5.1.7 Softwares

Software	Version	Company
CASY-messgerät	1.5	Schärfe System, D
Image J	1.52	NIH, USA
FlowJo	7.6.1	Tree Star, USA
GraphPad Prism	7.00	GraphPad Software, USA
Microsoft Excel	2016	Microsoft Corporation, USA
NCBI Databases		National Center for Biotechnology Information, USA
ND-1000	V3.7	Nanodrop, USA
Primer 3	4.0.0	Source Forge, USA
UCSC Databases		UCSC Genome Bioinformatics Group
R statistical platform		The R Foundation

5.1.8 Kits

Kits	Manufacturer
Histamine ELISA	Labor Diagnostika Nord, D
PGE_2 EIA	Cayman, USA
Multiscribe Reverse transcription kit	Applied Biosystems, Thermo Fischer Scientific, D
Multiplex ELISA Kit	Biolegend, Inc., USA
Nucleospin RNA II kit	Macherey-Nagel, D
Nucleospin plasmid kit	Macherey-Nagel, D
Rotor-gene SYBR green kit	Qiagen, GmBH, D

5.1.9 Buffers and Solutions

Buffer/Solution	Composition	Manufacturer
0.5N NaOH	10 g NaOH 500 ml water	
0.5M Tris-buffer	30.285 g tris-HCl 500 ml water	
1X Running buffer	100 ml 10X running buffer 900 ml water	
1X TBS	100 ml 10X TBS 900 ml water	
1X Transfer buffer	100 ml 10x transfer buffer 200 ml methanol 700 ml water	
1.5 M Tris buffer	90.855 g tris-HCl 500 ml water	
2mM PBS-EDTA	490 ml PBS 10 ml 100 mM EDTA	

5X Sample buffer	4% SDS 100 mM tris-HCL 20 % glycerol 200 mM DTT 0.2 % bromphenol blue 0.1 % β-mercaptoethanol	
1% Perchloric acid	10 ml perchloric acid 90 ml water	
5% Milk in TBST	10 g milk powder 200 ml 1X TBST	
10% APS	10 g APS 100 ml water	
10% SDS	10 g SDS 100 ml water	
10X Running buffer	144 g glycine 30 g trizma base 10 g SDS 1 l water	
10X TBS	121.1 g trizma base 90 g NaCl 1 l water	
10X TBST	1 l- 1x TBS 5 ml Tween20 10 l Water	
10X Transfer buffer	144 g Glycine 30 g Trizma Base 1 l Water	
50X PIPES, pH 7.4	1.1M NaCl 50 mM KCl 250 mM PIPES	
DMEM high glucose		Biochrom, D
FACS buffer	1% BSA/1X PBS	
IMDM		PAA, A

Basal Iscove's		Biochrom, D
Lysis buffer		
Medium BMcMCs	IMDM Medium 10% FCS 1% P/S 10 ng/ml IL-3 0.002% αMTG 30 ng/ml SCF	
Medium HuMCs	Basal Iscove's 10% FCS 1% Penicillin/ Streptomycin 2mM l-glutamine 1% non-essential amino acids 100 ng/ml hrSCF	
MACS buffer	0.2% BSA/1X PBS 20mM EDTA	
PBS pH 7.4		PAA, A
PBS with Ca^{+2} and Mg^{+2}		PAA, A
PBST	0.05% Tween/1X PBS	
PAG-CM	1X Pipes 0.1% Glucose 0-1% BSA	
Ponceau S	0.5 ml Ponceau S 25 ml Acetic Acid 500 ml Water	
Transport medium	IMDM-medium 10% P/S	

5.2 Methods

5.2.1 Cell Biology Methods

5.2.1.1 Mast cell isolation and culture

5.2.1.1.1 Human mast cells (HuMCs)

MCs were isolated from human breast skin or foreskin (cosmetic breast reduction surgery or circumcisions). MC isolation and purification was performed using an optimized and frequently employed protocol [194-196], giving rise almost to 98-100% pure MC preparations. The skin samples were obtained after written consent of patients or legal guardians and approved by the University Ethics Committee. Experiments were performed according to the Declaration of Helsinki Principles.

In brief, human skin was cut into strips and treated with dispase (3.5 U/ml) and 4 °C overnight. After removal of the epidermis, the dermis was chopped into small pieces and digested with collagenase type 1 (10 mg/ml) for 1 h at 37 °C. MC purification was achieved by positive selection with mouse anti-human c-Kit-coated microbeads and an Auto-MACS separation device. Skin-derived MCs were expanded in basal Iscove's Medium (with 10% FCS, 100 U/mL Penicillin, 100 µg/mL Streptomycin, 2 mM l-Glutamine, 1% nonessential amino acid solution, stem cell factor (100 ng/ml) and IL-4 (10 ng/ml) as described previously.[197] The cells were used for the experiments described after 3–5 weeks, when proliferation is detectable.[198] The vitality and purity of the cells was determined with trypan blue/toluidine blue staining using a light microscope and by using a cell counter *(CASY)*.

5.2.1.1.2 Murine bone-marrow derived cultured mast cells

Murine bone-marrow derived cultured mast cells (BMcMCs) were obtained from female C57BL/6J mice using a published protocol. [199] BMcMCs or MC-progenitor-cells are

immature and belong to the category of mucosal MCs. [200, 201] They are cultured in IMDM medium in the presence of mouse recombinant IL-3 for 4-6 weeks which promotes their growth and development (37ºC and 5% CO_2 in a humidified atmosphere). [17]

Femur and tibia from a mouse were isolated and cleaned of any tissue remnants. They were carried in 50 ml transport medium until further use. All further steps were carried out under a sterile airflow bench. The tips of bones were cut and with the help of a 25-gauge needle and culture medium the bone-marrow was washed out. A 10 ml pipette was used to resuspend the bone-marrow in fresh medium followed by centrifugation at 1300rpm, 4°C for 10 min in 20 ml medium. The recovered pellet was resuspended in 20 ml fresh culture medium and transferred to a 75 cm^2 culture flask. To separate adherent stromal cells from the suspension MCs, the cell culture medium and the flask was changed on Day 5, 8, 10 and 15 and transferred to a new flask. After day 19, the medium was changed twice a week, once completely and once 50% alternately. The growth factor mrIL3 was added after each medium change at an end concentration of 10 ng/ml. The vitality of BMcMCs was determined like HuMCs. The purity of BMcMCs was determined by the co-expression of c-Kit and FcεRI via FACS (section 3.2.4.3). Only cells with a purity >90 % were used for experiments.

5.2.1.2 Histamine release assay

5.2.1.2.1 Pre-treatment of human mast cells with PGE_2/EP agonists and the analysis of histamine release

Freshly isolated HuMCs were sensitized with IgE (0.5µg/ml) overnight without cytokines. Cells were washed with PAG-CM buffer (pH 7.5), containing 3 mmol/L $CaCl_2$ and 1.5 mmol/L $MgCl_2$ and treated with various concentrations of PGE_2 or EP agonists (ONO-AE1–259 (EP-2), ONO-AE-248 (EP-3), ONO-AE1–329 (EP-4), kindly provided by Ono Pharmaceutical Company Ltd., Osaka, Japan, for 30 min at 37ºC. Cells were stimulated by anti-human IgE antibody (0.5 µg/ml) for 25 min at 37ºC.

For the assessment of the spontaneous histamine release, cells were kept in buffer only. Total histamine content (complete) was determined after cell lysis with 1% perchloric acid. The degree of MC stimulability was assessed with calcium ionophore

A23187 (2 mmol/L). Cell supernatants were used to determine the histamine release using an autoanalyzer. Net histamine release was calculated as follows: Net histamine release (%) = [(Stimulated release-Spontaneous release)/Complete] *100.

5.2.1.2.2 Pre-treatment of murine mast cells with PGE_2/EP agonists and the analysis of histamine and cytokine release

BMcMCs were sensitized with IgE (1 mg/mL) for 48 h in complete media. Cells were washed with PAG-CM buffer (pH 7.5), containing 3 mmol/L $CaCl_2$ and 1.5 mmol/L $MgCl_2$ and treated with various concentrations of PGE_2 or EP agonists (ONO-AE1–259 (EP-2), ONO-AE-248 (EP-3), ONO-AE1–329 (EP-4) (alone/in combination) for 30 min at 37ºC. BMcMCs were stimulated by anti-IgE (5 µg/mL) for 25 min at 37ºC.

For the assessment of the spontaneous histamine release, cells were kept in buffer only. Total histamine content (complete) was determined after cell lysis with 1% perchloric acid. The degree of MC stimulability was assessed with calcium ionophore A23187 (2 mmol/L). Cell supernatants were used to determine the histamine release using an autoanalyzer. Net histamine release was calculated as follows: Net histamine release (%) = [(Stimulated release-Spontaneous release)/Complete] *100.

For the measurement of cytokines, BMcMCs were treated as above and stimulated with anti-IgE for 8 h at 37ºC.

5.2.2 Anaphylaxis in the murine system

5.2.2.1 Mice strains

Female Balb/c and C57BL/6J mice (10-12 weeks old) were obtained from Charles River (Sulzfeld, Germany). Animals were kept under specific pathogen-free conditions in a temperature-controlled environment with free access to standard chow and water. The local State Office of Health and Social Affairs approved all experiments (G0200/11, G0004/16). C57BL/6J mice will be referred to as BL/6 in the following.

5.2.2.2 Passive systemic anaphylaxis (PSA)

5.2.2.2.1 Comparison of anaphylactic susceptibility between Balb/c and BL/6 mice

To analyze the anaphylactic susceptibility in the 2 strains of mice, a PSA model [58, 202] to induce sub-optimal ANA in these mice was first established. For this purpose, the antibody and antigen concentrations were optimized. A consistent amount of anti-trinitrophosphate (TNP)-IgE (0.8 mg/kg, i.v.) was injected, followed by the injection of different doses of TNP-BSA, 24 h later. The systemic ANA reaction was evaluated by measuring the core body temperature.[58, 84] Changes in rectal temperature were assessed in 10 min intervals for 60 min using a digital thermometer.

After establishment of the method, mice were passively sensitized with mouse anti-TNP-IgE (0.8 mg/kg, i.v.) and challenged with an optimized dose of TNP-BSA (0.28 mg/kg, i.v.), 24 h later. Changes in rectal temperature were assessed. After the assessment, mice were euthanized, and serum samples were collected and stored at -80°C for further analysis.

5.2.2.2.2 15-PGDH inhibitor and the induction of PSA

Mice were sensitized with mouse anti-TNP-IgE (0.8 mg/kg, i.v.), on day 1, followed by the administration of 15-hydroxyprostaglandin dehydrogenase inhibitor (15-PGDH-I) 10mg/kg) on day 2. They were challenged by injection of TNP-BSA (0.6 mg/kg, BL/6; 4 mg/kg, Balb/c, i.v.), 2.5 h later (Fig. 7). Sham groups received DMSO (vehicle) as a negative control. Temperature and serum acquisitions were performed as described above.

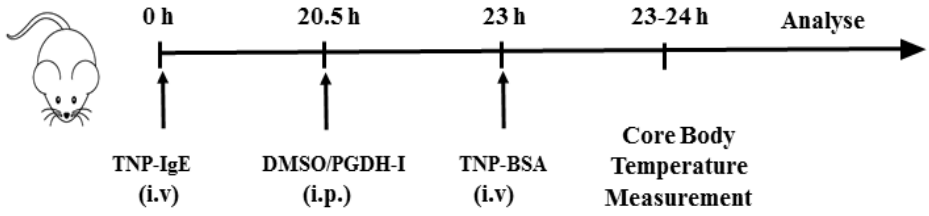

Figure 7: Experimental model for 15-PGDH-I treatment and the induction of ANA.

5.2.2.2.3 Acetylsalicylic acid and the induction of PSA

BL/6 mice were treated with ASA (50 mg/kg, o.g.) or PBS for different time periods for optimization before the induction of systemic ANA (Fig. 8). After the treatment of ASA, mice were sensitized with an injection of anti-TNP-IgE (0.8 mg/kg, i.v.) followed by TNP-BSA (0.28 mg/kg, i.v.) challenge, 24 h later. Temperature and serum acquisitions were performed as described above.

.

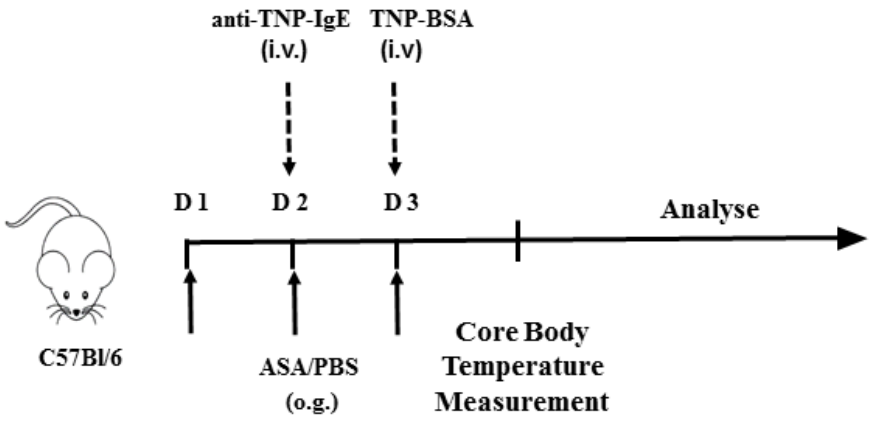

Figure 8: Experimental model with ASA treatment and the induction of ANA.

5.2.2.2.4 PGE_2/EP agonists/15-PGDH-I and acetylsalicylic acid-aggravated PSA

BL/6 mice were treated with ASA as described above and sensitized with an injection anti-TNP-IgE (0.8 mg/kg, i.v.) on day 2 (Fig. 9). Mice were treated with PGE_2/EP agonists/15-PGDH-I (0.3-7.5 µg/kg, PGE_2; 10 µg/kg, EP agonists; 10 mg/kg, 15-PGDH-I, s.c) or PBS (vehicle) on day 3, followed 45 min or 2.5 h (in case of 15-PGDH-I) later by TNP-BSA (0.14 mg/kg, i.v.) challenge for the induction of PSA. Temperature and serum acquisitions were performed as described above.

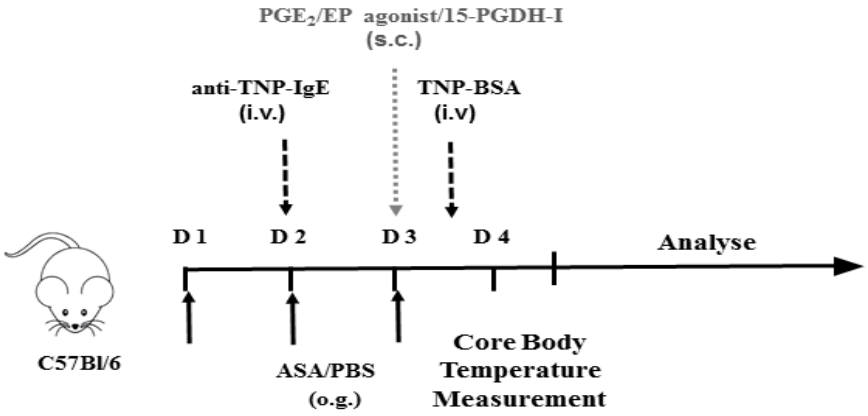

Figure 9: Experimental model with PGE_2/EP agonists/15-PGDH-I treatment and the induction of ANA.

5.2.2.2.5 COX-1/2 inhibitors and acetylsalicylic acid-aggravated PSA

BL/6 mice were treated with a COX-1 (SC560) or a COX-2 (celecoxib) inhibitor (20 mg/kg, o.g.) or PBS (vehicle) before the induction of systemic ANA. Animals were treated with COX-1/COX-2 inhibitor for 2 days. The last administration was given 24 h or 16 h before ANA induction. Mice were sensitized and challenged as described above (Fig. 10).

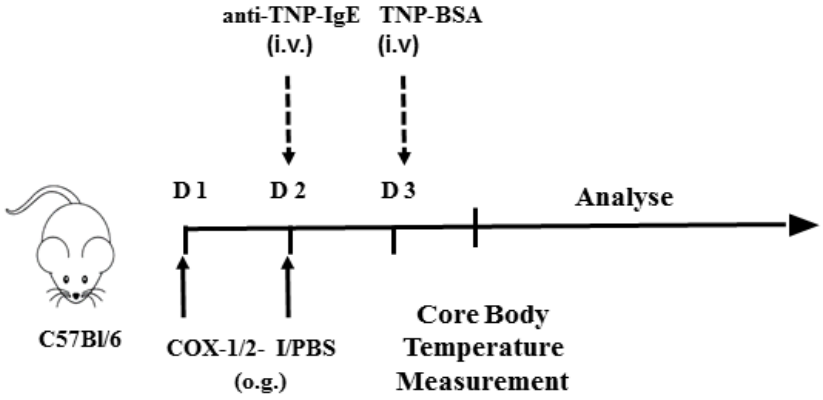

Figure 10: Experimental model for COX-1/2 inhibitor treatment and the induction of ANA.

5.2.3 Anaphylaxis patient study

Sera samples from 27 healthy subjects and 48 patients with a history of hymenoptera sting ANA were obtained from the outpatient clinic of the Department of Dermatology, Venereology and Allergy. The samples were defined into 4 groups according to their

ANA severity grades [Ring and Mesmer [70]]. Severity grading was based on the clinical reaction patterns of the most recent ANA episode recorded assessed before first aid treatment was given and most patients belonged to severity grades II and III. The average age of control subjects and patients was 40 yr. and 48 yr. respectively. All patients gave written consent and data/sample acquisition was approved by the Ethics Committee at Charité-Universitätsmedizin Berlin.

5.2.4 Analytical methods

5.2.4.1 Histamine measurement using an autoanalyzer

Histamine can be quantified using an autoanalyzer. [203, 204] This method is based on coupling of histamine with o-phthalaldehyde at a highly alkaline pH to form a fluorescent product. The fluorescence of histamine-o-phthalaldehyde complex is more stable at acidic pH. To remove interfering compounds, histamine is extracted before condensation. Protein is removed from the sample using perchloric acid precipitation; histamine is extracted into n-butanol and recovered in an aqueous solution of dilute HCl by adding heptane. The dilute HCl solution is used for the condensation of histamine with o-phthalaldehyde. The extraction procedure removes histidine and other interfering compounds before the condensation step. The fluorescence intensity of the histamine-o-phthalaldehyde complex is directly proportional to the histamine level in samples and is detected by a computer. By using histamine standards, the histamine concentration in samples can be determined.

5.2.4.2 Quantification of prostaglandins, mast cell mediators and cytokines by ‘Enzyme-linked immunosorbent assay’ (ELISA)

For the measurement of histamine and PGE_2 in serum, competitive ELISAs were used. These assays are based on the competition between the antigen in the samples and standards which are bound to the solid carrier or added, for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antibody-antigen complexes are removed by washing. The antibody bound to the solid carrier can be detected by an enzyme-conjugated secondary antibody using a substrate. The substrate is converted into a colored product and its absorbance is measured. The

intensity of the color developed is positively correlated to the amount of enzyme and inversely proportional to the concentration of the target molecule in the test sample. The quantification of samples is achieved by comparing their absorbance with values from the standard curve prepared with known concentrations.
For the detection of cytokines, BMcMCs were pre-treated as mentioned in section 6.2.1.2.2, but stimulated with anti-IgE for 8 h. The amounts of TNF-α, IL-6, IL-13, CCL-2 and CCL-3 were determined in supernatants following manufacturer's instruction using a multiplex ELISA.

5.2.4.3 Flow cytometry analysis of mast cells

Flow cytometry is an optical technique used for the analysis of single cells in a cell suspension. Cells are labelled with antibodies conjugated to fluorescent molecules called fluorochromes, which absorb light of a particular wavelength to emit light of another wavelength known as excitation and emission. Flow cytometry allows the detection and separation of a cell labelled with fluorochromes. This technique uses lasers to excite fluorochromes tagged to the cells, which emit light, recorded by a detection system. Since the cells pass one by one through the laser, the machine keeps count of cells and the level of fluorescence produced. For the detection of specific extracellular or intracellular proteins, cells can be marked and identified with different fluorochrome-conjugated antibodies. [205]

To determine the purity of BMcMCs, 500,000 cells were used per sample. They were washed with 1X PBS and centrifuged at 2400 rpm, 4°C for 10 min. Cells were incubated with FcR block for 15 min at 4ºC and subsequently stained with fluorochrome-labelled FcεRI-PE and cKIT-APC Cy7 antibodies against MC specific markers FcεRI (IgE receptor) and cKIT (KIT receptor) for 30 min at 4ºC. Antibody with the same isotype was used to stain control samples. Simultaneously cells were stained with DAPI to determine living cells. After the staining, cells were washed and measured with the *MACSQuant* Analyzer. The data was analyzed using *FlowJo* software. Cells double positive for both FcεRI and cKIT were considered MCs.

5.2.5 Molecular Biology Methods

To determine EP receptors' expression, a quantitative real time polymerase chain reaction (qRT-PCR) was performed. Ribonucleic acid (RNA) was isolated from human/murine MCs and transcribed into complementary DNA (cDNA) as a template for the qPCR reaction.

5.2.5.1 Isolation of ribonucleic acid

$1*10^6$ cells (BMcMCs or HuMCs) were washed with 1X PBS and centrifuged at 1300 rpm, 4°C for 10 min. The pellet was resuspended in 350 µl lysis buffer (*RA1, NucleospinRNA II*) with 3.5 µl β-mercaptoethanol (β-ME). The RNA isolation was performed according to protocol of the Nucleospin RNA II kit. RNA was eluted with 30 µl RNAse-free water passed through the column two times.

5.2.5.2 Synthesis of complementary cDNA

Synthesis of cDNA was performed using *Taqman reverse transcription* reagents, with a *multiscribe reverse transcriptase* enzyme. Maximum 1µg RNA was taken in a volume of 7.7 µl with 12.3 µl reaction mix (Table 2) and incubated in a thermocycler according to the protocol of cDNA synthesis. cDNA was stored at -20°C until further usage.

Reagent	**Volume (µl)**	**Endconcentration**
10x Taqman RT buffer	2.0	1x
25 mM $MgCl_2$	4.4	3-6 mM
10 µM deoxyNTPs	4	2 µM
50 µM RandomHexamer primer	0.5	1.25 µM
50 µM Oligo $d(T)_{16}$ Primer	0.5	1.25 µM
20 U/µl RNAse Inhibitor	0.4	0.4 U/µl
50 U/µl MultiScribe reverse transcriptase	0.5	1.25 U/µl

Table 2: Reaction mix for Reverse Transcription

5.2.5.3 Quantitative polymerase chain reaction (qPCR)

qPCR is performed to analyze the expression of genes using cDNA as a template. This technique uses sybr green, a fluorescent double-stranded DNA intercalating dye, that fluoresces once bound to the DNA. A pair of gene-specific primers are used to amplify the template DNA. The amount of dye incorporated is directly proportional to the amount of product formed. The dye emits at 520 nm and fluorescence emitted can be measured and related to the amount of product formed. The DNA template was diluted 1:3 and mixed with specific primers and sybr green mix from *Quantitech SYBR green PCR kit.* The reaction mix (Table 3) was then incubated in the Rotor gene Q (QIAGEN GmbH, Hilden, Germany).

Reagent	**Volume (µl)**	**End Concentration**
2x Rotor-Gene SYBR Green PCR Master Mix	5	1x
10 µM Primer, forward	1	1 µM
10 µm Primer, reverse	1	1 µM
H_2O	1	

Table 3: Reaction mix for a qPCR reaction

All primers used (Table 4) were oligonucleotide primers designed with the help of the *Primer3* software and purchased from TIB Molbiol, Berlin, Germany. Specific conditions for each primer are mentioned in the table below:

Gene	**Sequence (5'-3')**	**Size (bp)**	**Conditions** $T_{Den}/T_{An}/T_{Ex}$ $t_{Den}/t_{An}/t_{Ex}$
TBP	for: TTTGTGCCAGATACATTCCG rev: AACAATTTACAAGCTGCGTTT	127	95/60/72 10/10/10
mEP2	for: GACGGACCACCTCATTCTC rev: CTAAGTATGGCAAAGACCCAAG	180	95/65/72 10/10/10
mEP3	for: ATGATGGTCACTGGCTTCGT rev: ATGGTTAGCCCGAAGAAGGT	239	95/60/72 10/10/10

mEP4	for: ATCTTACTCATCGCCACCTCTC rev: ATCTGGGTTTCTGCTGATGTCT		95/65/72 10/10/10
hHPRT	for: CCTGGCGTCGTCATTAGTGA rev: GCCTCCCATCTCCTTCATCA	162	95/65/72 10/10/7
hEP2	for: CCACCTCATTCTCCTGGCTA rev: CGACAACAGAGGACTGAACG	216	95/63/72 10 /10/5
hEP3	for: GGATCATGTGCGTGCTGTC rev: TGCTTCTCCGTGTGTGTCTT	109	95/63/72 10/10/5
hEP4	for: TCATCTTACTCATTGCCACCTC rev: ACTGACTTCTCGCTCCAAACT	110	95/63/72 10/10/5
mCCL-2	for: AGCACCAGCCAACTCTCACT rev: TCATTGGGATCATCTTGCTG	184	95/58/72 10/10/5
mCCL-3	for: CAGCCAGGTGTCATTTTCCT rev: CTCAAGCCCCTGCTCTACAC	170	95/62/72 10/10/5
mIL-13	for: CAGCTCCCTGGTTCTCTCAC rev: CCACACTCCATACCATGCTG	212	95/58/72 10/10/5
mIL-8	for: ATGCCTCTCCATTTCCTGCT rev: CATGGGGAAAGAGGCTCTGA	155	95/60/72 10/10/5
TNF-α	for: CCACCACGCTCTTCTGTCTA rev: GGTTGTCTTTGAGATCCATGC	198	95/60/72 10/10/5

Table 4: Primer sequences for qPCR

The expression levels of the target genes were quantified relative to the expression of a reference gene using the 2-ΔΔCT method.

5.2.5.4 Transfection

HEK 293S cells were stably transfected with EP2-4 cDNA clones to generate positive controls for studying EP receptor expression in MCs.

5.2.5.4.1 Clone preparation

Empty pTCN vector and pTCN-EP receptor 2-4 cDNA clones were purchased as glycerol stocks from *transOMIC Technologies*. The pTCN vector has a CMV promoter and resistance for prokaryotic and eukaryotic systems (carbenicillin/neomycin). The details of this vector can be seen in Fig. 11.

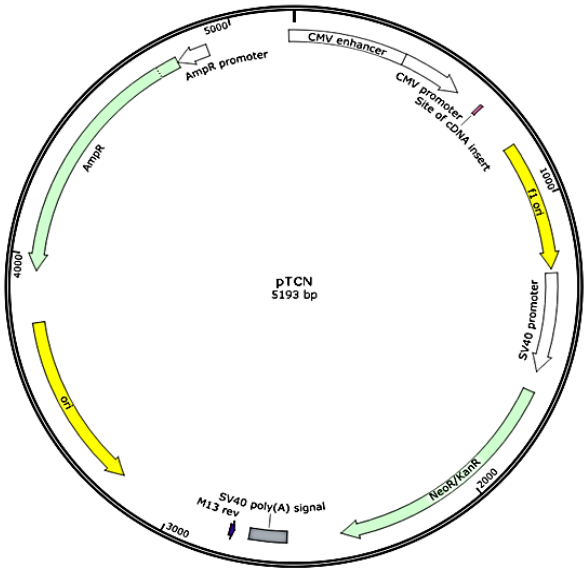

Figure 11: Detailed map of pTCN vector (transOMIC technologies). pTCN is a 5193 bp long high copy plasmid with a CMV promoter for cDNA expression. It has a poly A element to provide polyadenylation signal for mRNA stabilization and SV40 promoter to drive selective marker expression. The selection marker provided in this vector is neomycin and has pUC ori for propagation in E. coli. Ampicillin is the bacterial selection marker to be used.

For the amplification of cDNA clones (Fig. 12), 200 µl pre-warmed LB medium was inoculated with the glycerol stocks and spread on LB agar plates containing carbenicillin (100 µg/ml). After overnight incubation at 37°C, single colonies appeared on the plate. Three single colonies were used to inoculate three, 5 ml LB broth starter cultures containing 5 µl carbenicillin. After overnight incubation at 37°C with shaking, these cultures were used for plasmid purification according to the instructions using NucleoSpin Plasmid kit. The potential clones were confirmed using double restriction digestion and sequencing. Restriction digestion was performed using enzymes Xba I and Bgl II. 1 µg DNA was incubated with 4 µl master mix according to instructions for the enzymes and products were visualized using a 1% agarose gel. Clones were confirmed by sequencing with the help of *Eurofins Genomics.*

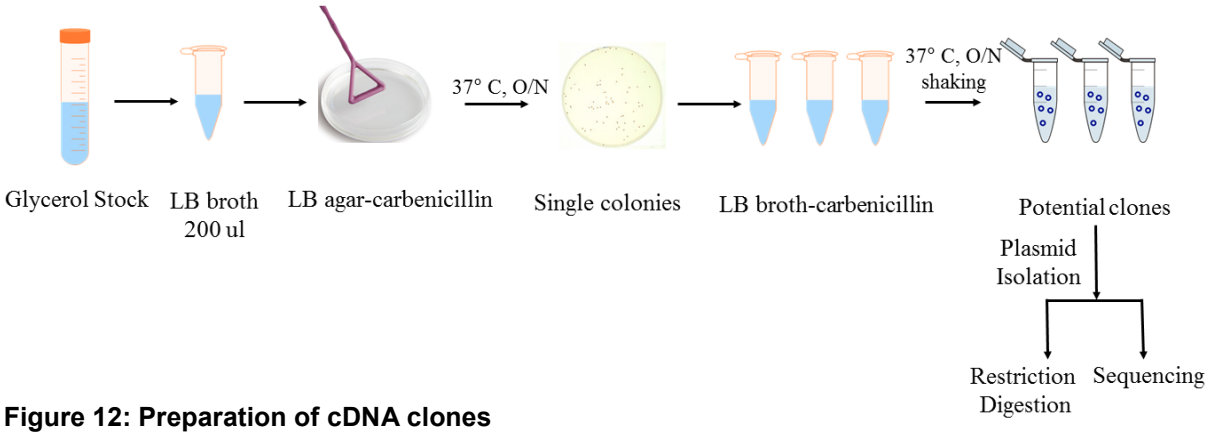

Figure 12: Preparation of cDNA clones

5.2.5.4.2 Stable transfection

$2.5*10^4$ HEK 293S cells/well were plated in 1 ml DMEM high glucose complete medium in a 12-well cell culture plate and incubated for 24 h at 37°C in a CO_2 incubator such that they are 50% confluent after 24 h. Cells were washed with 1x PBS and the media was replaced with 950 µl antibiotic-free DMEM high glucose media. Cells were transfected using *Fugene-6* transfection reagent. To prepare the transfection complex two complexes were prepared. Complex 1 included 1µg DNA in 25 µl antibiotic-free DMEM medium. Complex 2 contained 3 µl Fugene-6 transfection reagent in 22 µl medium. The two complexes were incubated together for 15-20 min after which the mix was added to the cells drop-wise with shaking. The cells were incubated for 48 h at 37°C in a CO_2 incubator. After 48 h, cells were washed with 1x PBS and 1 ml complete DMEM medium was added together with 1 mg/ml G418. Cells were fed every 3 days for 2.5 weeks. Cells were serially diluted in 100 µl complete medium in a 96-well plate to attain limiting dilution. Single cell clones were expanded.

Stably transfected HEK 293S-EP2, HEK293S-EP3, HEK293S-EP4 cells were checked for the enhanced expression of EP receptors by using qPCR (section 6.2.5.3) and immunoblotting (section 6.2.5.5).

5.2.5.5 Immunoblotting (IB) and Immunoprecipitation (IP)

IB was used to analyze the expression of EP receptors and the phosphorylation of proteins in MCs.

EP receptor expression

To establish the protocol and test the antibodies for EP receptor expression, HEK293 cells stably transfected with EP receptors were used as positive controls. In order to isolate EP 2-4 receptor proteins from cell lysate, IP was used prior to detection by IB.

IP

IP is a technique used to isolate a specific antigen from a mixture, using antigen-antibody interaction. Antigens isolated are then analyzed by IB. Around 30-35*10^6 HEK-EP (2-4) overexpressed cells (~900µg-1mg protein) were lysed in 1 ml of lysis buffer. Protein G magnetic beads (0.25 mg) were washed and separated using a magnetic stand. Beads were then incubated with specific antibody (anti-EP 2-4, 5 µg)

together with wash buffer (1 ml) and incubated for 2-4 h, at 4°C with mixing. The magnetic beads were then separated using a magnetic stand and combined with the cell lysate together with the wash buffer (1 ml). The lysate was incubated with an irrelevant antibody as a negative control. The mixture was incubated overnight at 4°C with mixing. The beads were collected again using a magnetic stand and the flow-through was removed (collect for analysis). The magnetic beads were washed 3x with 500 µl of wash buffer and separated using a magnetic stand. The supernatant was removed carefully. 500µL of ultrapure water was added to the tube and gently mixed. The beads were collected discarding the supernatant. The wash was repeated twice. EP proteins were eluted by adding 50 ul SDS sample buffer (1X) and heating for 10 min to 95°C. The beads were magnetically separated and the supernatant for further used for Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

IB

To detect EP receptors, eluted proteins were separated through a 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the proteins were transferred to PVDF membranes. The membranes were blocked with 5% milk and incubated with corresponding primary antibodies (Cayman/Santa Cruz/Atlas) overnight and subsequently with (1: 7500 diluted) HRP (horseradish peroxidase)-conjugated secondary antibody for 1h. Finally, blots were visualized by a chemiluminescence assay (ECL) according to the manufacturer's instructions, and the bands were recorded using a detector for chemiluminescence. To quantitate changes in expression blots images were quantified using ImageJ (1.48v). Arbitrary values were determined using the following equation:

$$\text{Relative target expression} = \frac{\text{density}_{\text{target}}}{\text{density}_{\text{housekeeping gene}}}$$

Protein levels were normalized to β-actin by using densitometry. Images were cropped. The conditions shown in Table 5 were used to optimize the detection of EP receptors 2-4 using IP+/- IB.

Technique	Reagents	Condition/s
IB	Antibodies from Cayman Chemicals	Titration (EP2-1:200-1:20000, EP3-1:200-1:2000, EP4- 1:200-1:2000) with different concentrations of protein. (10, 60µg, 100µg, 150µg)
IB	Antibodies from Santa Cruz (S/C)	Titration (EP2-1:100-1:2000, EP3- 1:200-1:1000, EP4- 1:200-1:1000).
IP+IB	Combinations of Cayman and S/C antibodies, Protein G beads	Cayman Ab+ S/C Ab, Cayman Ab +Cayman Ab, S/C Ab+ Cayman Ab, S/C Ab+ S/C Ab, Cayman + only secondary Ab, S/C Ab+ only secondary Ab
IB	Antibodies from Atlas Antibodies	Titration for EP3/4 with Atlas antibodies.

Table 5: Summary of conditions used to optimize the detection of EP receptor 2-4 expression in HEK293-EP 2-4 overexpressed cells. IP, immunoprecipitation; IB, immunoblotting.

Protein phosphorylation

To check the expression of phosphoproteins in BMcMCs, cells were sensitized with mIgE (1mg/mL) for 48 h, washed and resuspended in PAG-CM buffer. They were pre-incubated with 0.5/1/10 ng/ml of PGE_2 for 2 min and stimulated with anti-IgE (0.5 µg/mL) for an additional 15 min at 37ºC. The reaction was stopped with ice-cold 1X PIPES buffer and the cell pellet was processed for lysis. MCs were lysed and separated through 10% SDS-PAGE. The transfer, blotting and development procedures were same as mentioned above.

5.2.5.6 Statistical analysis

Paired samples in two groups were analyzed using a paired t-test (normal distribution) or the Wilcoxon matched-pairs signed rank test (non-parametric). Unpaired samples were analyzed using an unpaired t-test or Mann-Whitney rank test (non-parametric). Multiple experimental groups were analyzed by ANOVA/Kruskall Wallis test followed by Dunn-Sidak post-test. The core body temperature measurements in the PSA model were analyzed by 2-way ANOVA followed by a Sidak's test. Figures 15A and 17A were

analyzed by comparing the delta of area under the curve using Wilcoxon rank sum test with continuity correction. A p-value of 0.05 was considered statistically significant. Statistical analyses were performed using GraphPad software (version 7.0; GraphPad Software, La Jolla, Calif) and R Statistical Platform (206).

6. RESULTS

6.1 Relative lack of PGE_2 pre-disposes to anaphylaxis

6.1.1 Anaphylaxis patients display low levels of serum PGE_2

Compromised production of PGE_2 has been linked to the aggravation of rhinitis and asthma in patients of ASA-induced hypersensitivity. [170, 171] Other evidence regarding the role of COX inhibition in allergy and asthma also highlights PGE_2 as a possible cofactor. [135, 165] In order to study any alterations in PGE_2 levels associated with ANA, we analyzed sera samples from 27 healthy donors and 48 patients with a history of hymenoptera sting ANA (ANA-prone) for their levels of PGE_2 (section 5.2.3). ANA patients exhibited substantially lower levels of the lipid mediator in comparison with healthy controls (324.7 ± 33.73 pg/ml vs 142.3 ± 14.11 pg/ml) (Fig. 13A). The correlation of PGE_2 serum levels with the severity of the anaphylactic reaction in these patients revealed a tendency of an inverse correlation (r= -0.33) between PGE_2 values and the severity of the reaction (Fig. 13B).

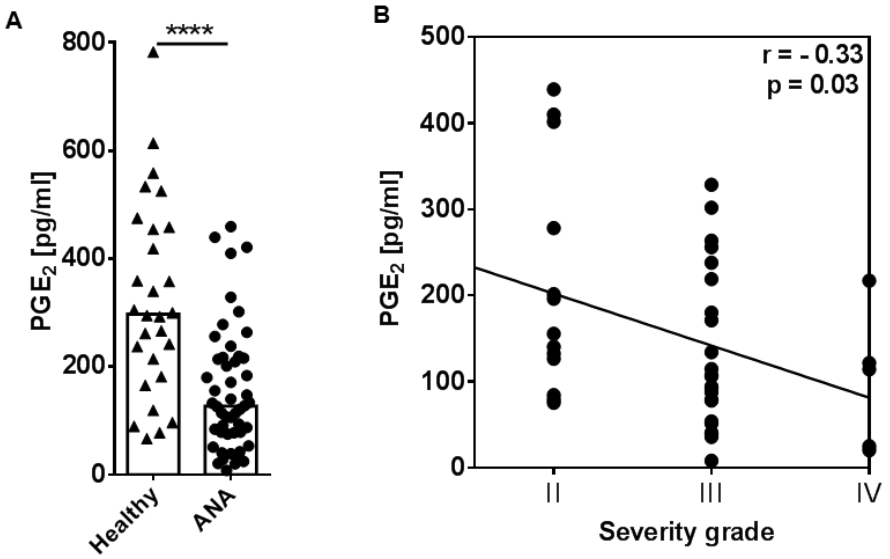

Figure 13: Anaphylaxis is characterized by PGE_2 deficiency. A) Serum levels of PGE_2 in sera of healthy control subjects (27) compared to ANA patients (48; outside of acute reaction), measured by ELISA. The data are shown as dot plots where each dot corresponds to one individual and median

depicted by column. B) Serum levels of PGE_2 as a function of severity of the most recent anaphylaxis episode recorded. ****P<0.0001.

6.1.2 Comparative analysis of anaphylaxis using BL/6/Balb/c mice

To study the role of a given genetic background in ANA, we compared two strains of mice frequently used as models of allergic disease, BL/6 and Balb/c. Animal studies investigating allergic diseases have shown these strains to differ in their immune responses. [207-211] A PSA model was used to compare the severity sub-optimal ANA in these mice. For this purpose, the dose of antigen was optimized. Mice were injected intravenously with a consistent amount of anti-TNP-IgE (0.8mg/kg), 24 h after which, both strains of mice were challenged with different doses of TNP-BSA. As a measure of systemic ANA, the core body temperature was measured every 10 min for a period of 60 min and plotted against the dose of antigen. As shown in figs. 14A and 14B for Balb/c and BL/6 mice respectively, the anaphylactic reaction (core body temperature versus time) follows a Gaussian distribution curve.

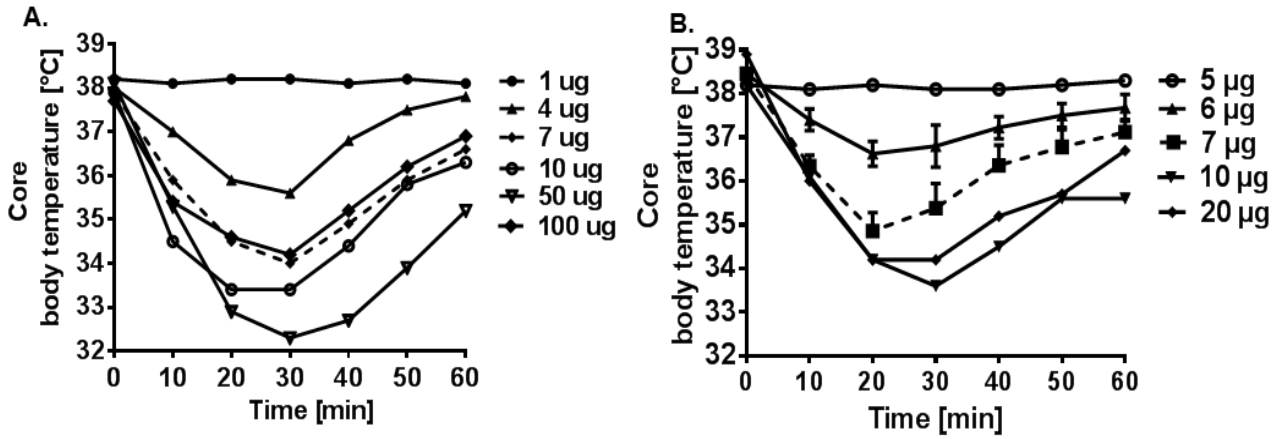

Figure 14: Dose response curves for TNP-BSA. Female A) Balb/c mice B) C57BL/6J mice were used to induce ANA with different doses of TNP-BSA. Rectal temperature assessment is shown as a function of time. (A, n=1/group; B, n=1/group [n=3 {6 µg, 7µg}])

The intensity of the anaphylactic reaction (reflected by the drop in core temperature), increased with increasing doses of the allergen reaching a maximum of 5.8°C at 50µg in Balb/c mice and 4.6°C at 10µg in BL/6 mice and decreasing thereafter. In Balb/c mice, 4µg of allergen elicited a temperature drop of 2.4°C; 7µg TNP-BSA caused a drop of 3°C. The maximal temperature drop was observed using 50µg TNP-BSA. On

the contrary, in BL/6 mice, 5 µg allergen resulted in a drop of 0.2°C and increased to 3.6°C with 7µg of allergen, reaching a maximum of 4.7°C with 20µg allergen. A temperature drop of 3-4°C is sufficient, to observe the effect of cofactors of ANA, therefore, a dose of 7µg TNP-BSA was used in all further PSA experiments.

6.1.3 BL/6 display a higher susceptibility towards anaphylaxis than Balb/c mice

Balb/c and BL/6 mice are two commonly used mouse strains to study hypersensitivity reactions like asthma or ANA. They are known to vary in the intensity of allergic responses. [207-213] A comparison of the severity of ANA between the two mouse strains, show a clear difference when using PSA. BL/6 mice exhibited a higher drop in core body temperature (Fig. 15A) when treated with similar dose of allergen (7µg) than Balb/c mice. This higher drop of temperature was accompanied by higher levels of serum histamine (Fig. 15B) indicating an increased response of the effector cells in BL/6 compared to Balb/c mice. The drop in core body temperature is used as a readout for anaphylactic reaction in the PSA model and was confirmed by the increased histamine levels.

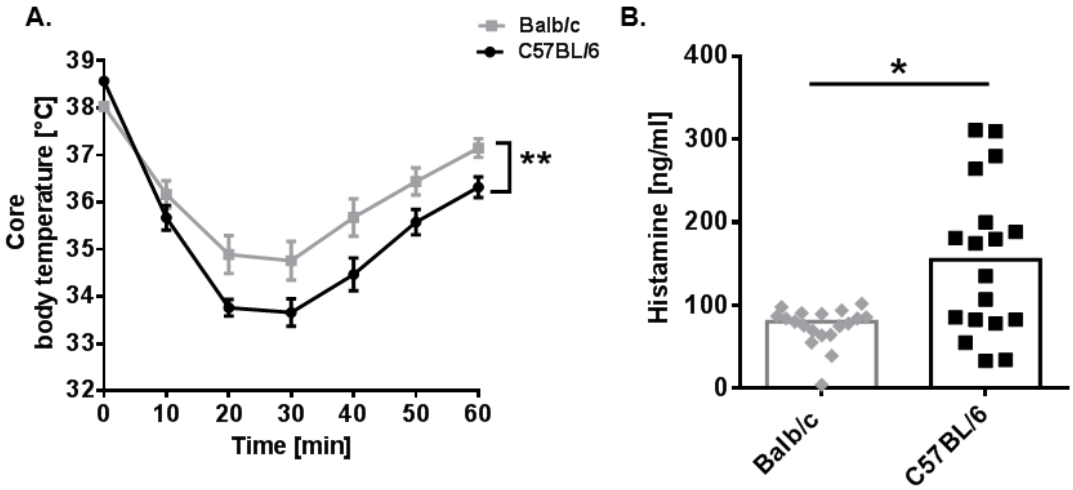

Figure 15: C57BL/6J mice develop more severe anaphylaxis than Balb/c. A) Core body temperature assessment shown as a function of time. The data are given as mean ± SEM, n=10-12. B) Serum levels of histamine upon elicitation of anaphylaxis, as determined by ELISA. The data are shown as dot plots with the median depicted as column. *P< 0.05, **P<0.01.

6.1.4 BL/6 mice display lower levels of serum PGE_2

An impaired production of PGE_2 has been reported to play a role in ASA-induced hypersensitivity reactions.[159, 170, 172] To investigate whether differences in PGE_2 levels are responsible for the variation in ANA intensity, two mouse strains were compared regarding their basal PGE_2 serum concentrations. We found significantly lower levels of PGE_2 in BL/6 mice (154.9 ± 21.46 pg/ml) as compared to Balb/c (494.4 ± 63.67 pg/ml) (Fig. 16). The data suggests that variation in PGE_2 levels among genotypes may contribute to the differential susceptibility of the strains towards anaphylactic reactions.

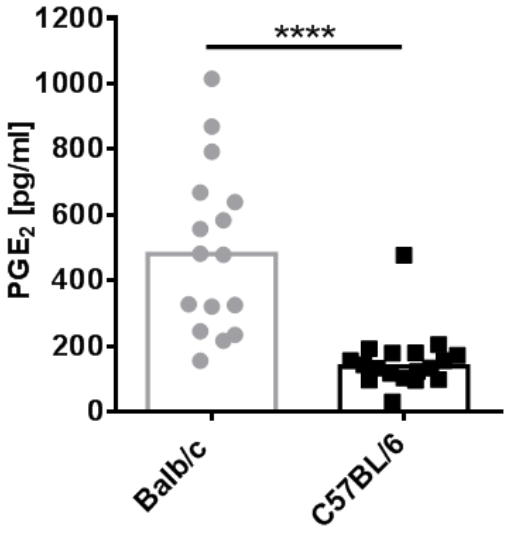

Figure 16: C57BL/6J display lower levels of PGE2 in comparison to Balb/c mice. Serum levels of PGE_2 at baseline were determined by ELISA and compared between Balb/c and C57BL/6J mice. The data are shown as dot plots with the median depicted as column. n= 16-17. ****P<0.0001.

6.1.5 Elevation of PGE_2 inhibits PSA in BL/6 mice

The above data suggest that the reduced PGE_2 may be relevant for the outcome of the ANA severity. To confirm this hypothesis, ANA-prone BL/6 mice were treated with an inhibitor of the 15-PGDH enzyme, responsible for PGE_2 degradation, which leads to a stabilization of PGE_2. By using this approach, we were indeed able to raise PGE_2 serum levels in BL/6 mice (data not shown). The inhibitor reverted the drop in core body temperature and resulted in faster recovery from ANA in BL/6 mice (Fig. 17A). This observation was accompanied by attenuated histamine release (Fig. 17B), matching the outcome of core body temperature. The inhibitor did not increase PGE_2 levels in Balb/c mice suggesting a low 15-PGDH activity. We were not able to

determine any effect on the development of ANA. However, more animals are needed to confirm these results (Figs. 17C/D). Taken together, these findings suggest that ANA susceptibility in BL/6 mice is at least partially influenced by PGE_2, whose augmentation can protect against ANA.

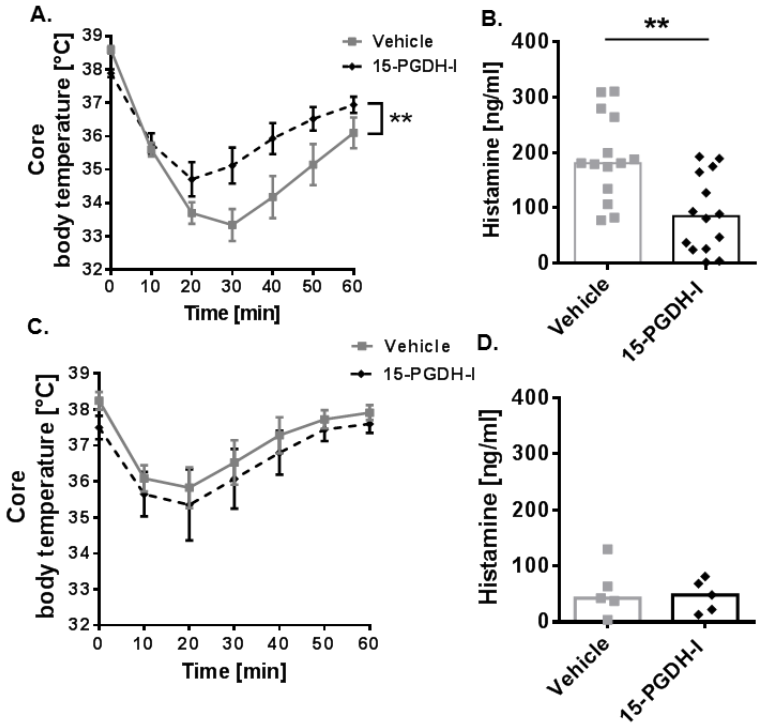

Figure 17: Stabilization of PGE_2 inhibits anaphylaxis in C57BL/6J mice. A-B) C57BL/6J and C-D) Balb/c mice were treated with 15-PGDH-inhibitor or vehicle, prior to PSA induction. Core body temperature measurement is shown as a function of time. The data are shown as mean ± SEM, A, n=6-8; C, n=4-7 B/D) Serum histamine levels upon elicitation of anaphylaxis were determined by means of ELISA. The data are shown as dot plots with the median depicted as column. **P<0.01.

6.2 PGE_2 decreases ASA-mediated aggravation of anaphylaxis

6.2.1 Acetylsalicylic acid aggravates PSA in BL/6 mice

As shown in our *in vivo* murine experiments in section 7.1.3, BL/6 mice are more sensitive towards developing anaphylactic reactions and are therefore most likely a better choice to study ANA in comparison to Balb/c. Our results suggest that low basal levels of PGE_2 in BL/6 mice pre-dispose to ANA, and an increase of endogenous PGE_2 alters the outcome of the reaction. Therefore, to further validate the protective role of

PGE_2 in ANA, BL/6 mice were treated with ASA, a well-known inhibitor of cyclooxygenases, and thereby the production of PGE_2.

To study the impact of ASA in the PSA model, BL/6 mice were treated with ASA or PBS (vehicle) as a negative control for different time durations (3 days until 24 h, 16 h or 90 min before the induction of systemic ANA) for optimization. Mice were treated with ASA before the induction of systemic ANA and the core body temperature was measured. Treatment with ASA aggravated the drop in core body temperature (5.9°C) in comparison to the vehicle-treated mice (4.3°C) (Fig. 18). The maximal aggravation in temperature drop (2.1°C) was detected in mice treated with ASA for 3 days with the last administration 90 min prior to the induction of ANA. Altogether, the data indicates that ASA aggravates hypothermia and hence ANA in BL/6 mice.

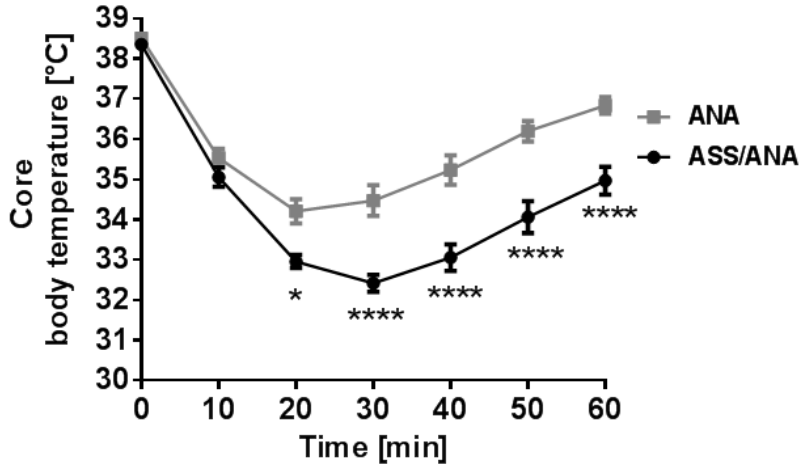

Figure 18: ASA aggravates anaphylaxis in C57Bl/6J mice. Female C57BL/6J mice were treated with ASA, (or PBS as a negative control) and PSA was induced. Core body temperature measurements are shown as a function of time. The data are shown as mean ± SEM. n=16-17. *P< 0.05, ****P<0.0001 (ANA; PBS-treated versus ANA/ASA; ASA-treated).

6.2.2 PGE_2/EP agonists 2/3 ameliorate ASA-mediated aggravation of anaphylaxis

To validate the protective impact of PGE_2 on ASA-aggravated ANA, mice were treated with ASA followed by the administration of various doses of PGE_2 before the induction of systemic ANA. PGE_2 was administered 90 min before the induction of ANA and the core body temperature was measured. PGE_2 treatment reduced the drop of the core body temperature caused by ASA from 5.5°C to 2.4°C, hence protecting against the exacerbation of ANA by ASA (Fig. 19A). Importantly, not only the administration of exogenous PGE_2 protected against the severity of ANA, the stabilization of endogenous PGE_2 by means of 15-PGDH-I similarly reduced ANA severity (5.95°C to 4.85°C) (Fig. 19B). These observations confirm our hypothesis that an increase of PGE_2 can protect mice against the severity of ANA.

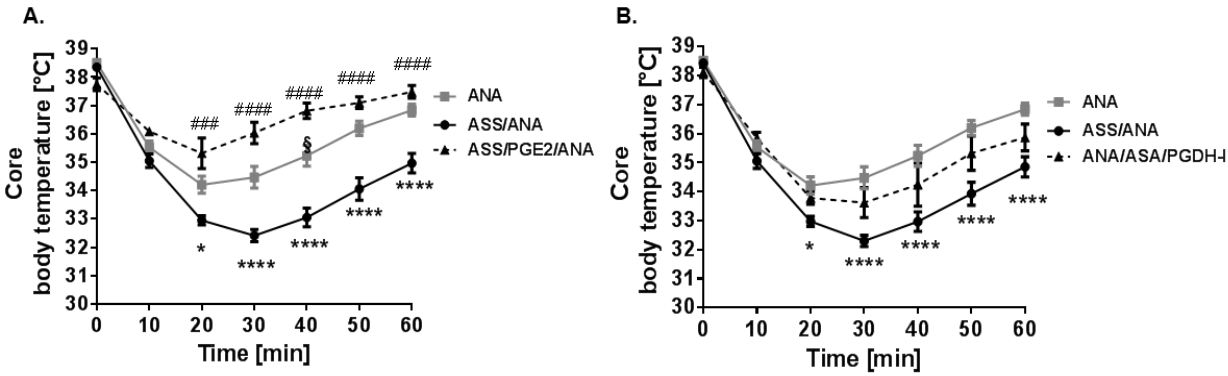

Figure 19: PGE_2 suppresses the potentiation of anaphylaxis by ASA. Female C57BL/6J mice were treated with ASA and A) PGE_2 B) 15-PGDH-I, (or PBS as a negative control) and PSA was induced. Core body temperature measurements are shown as a function of time. Data are shown as mean ± SEM. n=6-16 per group. *P< 0.05, ****P<0.0001 (ANA; PBS-treated versus ANA/ASA; ASA-treated); ###P<0.001, ####P<0.0001 (ASA/PGE2/ANA; ASA+PGE_2-treated versus ANA/ASA); §P<0.05 (ASA/PGE_2/ANA versus ANA).

PGE_2 exerts its effects via different EP receptors. EP2, EP3 and EP4 have been described previously to be broadly expressed throughout the body [214, 215] and to exhibit distinct actions in the context of allergy and asthma. [156, 216] To pinpoint the EP receptor responsible for the protective effect of PGE_2, mice were treated with ASA, followed by the administration of specific EP 2-4 receptor agonists [166, 217-220], 45 min before the induction of ANA. Treatment with EP2 agonist as expected reduced hypothermia (6.48°C to 4.37°C) preventing the aggravation of ANA by ASA (Fig. 20A). However, the effect of EP4 agonist was not significant (6.48°C to 5.66°C) despite a

slight tendency for protection (Fig. 20C). In contrast, the EP3 agonist was most potent (6.48°C to 4.19°C), reducing the aggravation of ANA by ASA (Fig. 20B). Overall, the data indicates that PGE_2 mediates its protective effect through EP receptors 2-4.

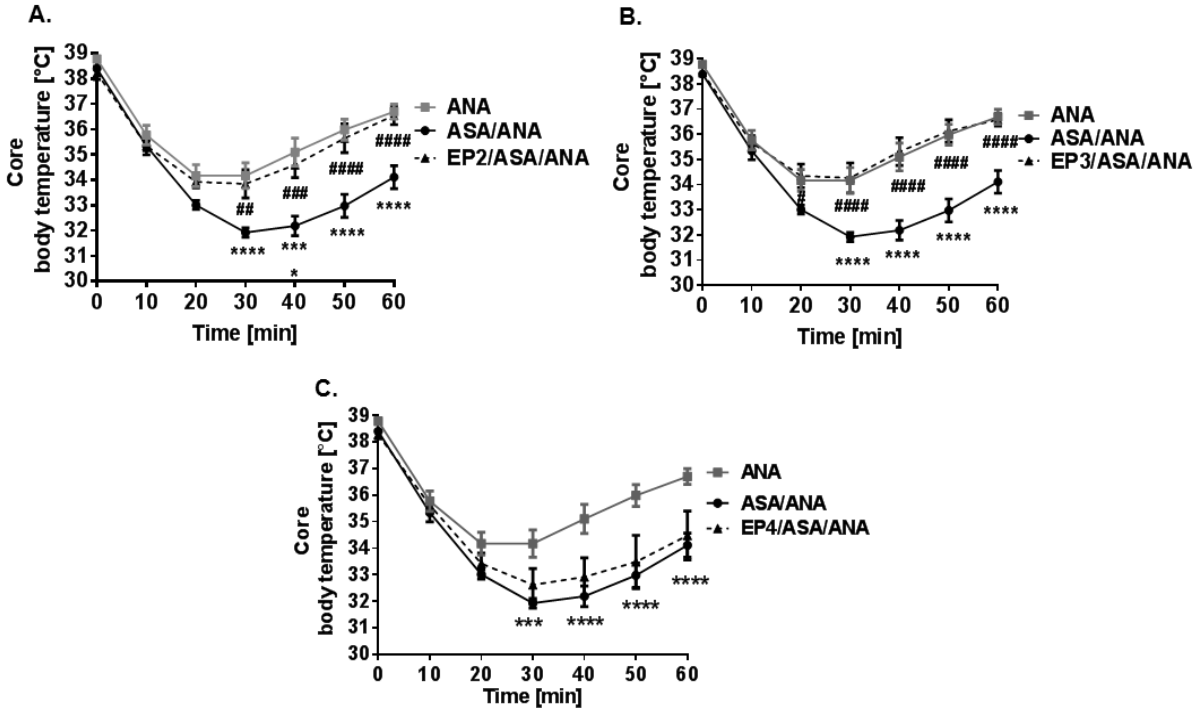

Figure 20: EP-Agonists moderate aggravation of anaphylaxis by ASA. Female C57BL/6J mice were treated with ASA and A) EP2 agonist B) EP3 agonist C) EP4 agonist (or PBS as a negative control), and PSA was induced. Core body temperature measurements are shown as a function of time. The data are shown as mean ± SEM. n=5-8 per group. ***P< 0.001, ****P<0.0001 (ANA; PBS-treated versus ANA/ASA; ASA-treated); ##P<0.01, ###P<0.001, ####P<0.0001 (EPX/ASA/ANA; EPX agonist-treated versus ASA/ANA).

6.2.3 COX-1/2 inhibitors aggravate PSA in BL/6 mice

ASA/NSAIDs act by inhibiting enzymes known as cyclooxygenases which exist in two isoforms namely COX-1 and COX-2. [133] To determine which COX (COX-1/2) subset is responsible for the impact of ASA on ANA in BL/6 mice, mice were treated with specific COX-inhibitors. SC-560 was used for COX-1 and celecoxib for COX-2 for 2 days. The last administration was given 24h before the induction of ANA and the core body temperature was measured. Both COX-1/2 inhibitors increased hypothermia in mice (COX-1: 5.9°C; COX-2: 6.2°C), almost to a similar degree as caused by ASA (6.5°C). The effect of COX-1 inhibitor was rapid reaching significance with 5.4 °C after 20 min and disappeared earlier (Fig. 21A), whereas the COX-2 inhibitor virtually mimicked ASA, reaching significance after 30 min (Fig. 21B). These results suggest

that the impact of ASA on systemic ANA is related to the inhibition of both COX-1 and COX-2 enzymes.

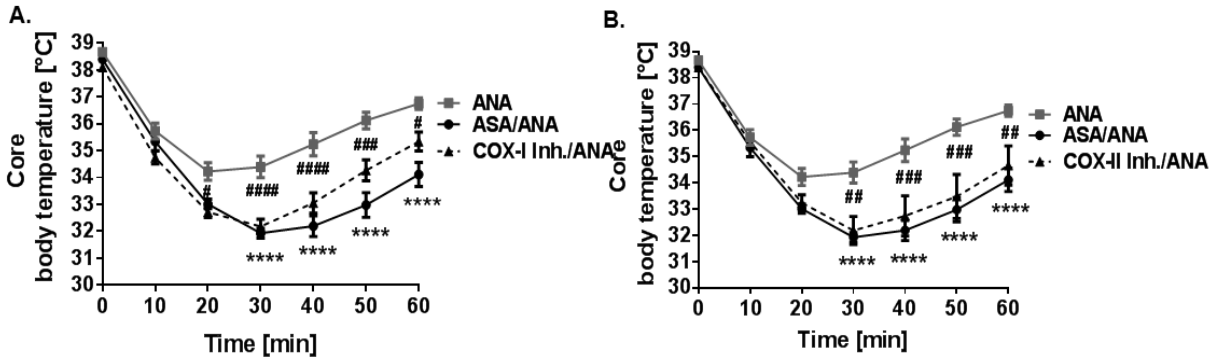

Figure 21: COX-1/2 inhibitors aggravate anaphylaxis. Female C57BL/6J mice were treated with A) COX-1 inhibitor B) COX-2 inhibitor, (or PBS as a negative control) and PSA was induced. Core body temperature measurements shown as a function of time. The data are shown as mean ± SEM. n=8-11, ****P<0.0001 (ANA; PBS-treated versus ANA/ASA; ASA-treated); #P<0.05, ##P<0.01, ###P<0.001, ####P<0.0001 (ANA versus ANA/COX-1/2; COX-1/2-treated).

6.3 PGE_2 partially reduces anaphylaxis via its effect on mast cells

6.3.1 PGE_2 reduces mast cell activation in BL/6 mice unlike Balb/c-derived cells

MCs release preformed as well as *de novo* synthesized mediators and cytokines, upon activation. To pinpoint the mechanism of PGE_2-mediated amelioration of ANA and find out if MC responses are differently regulated in these strains, BMcMCs from BL/6 and Balb/c mice were used to study MC responses towards PGE_2. Cells were treated with various concentrations (0.05-10 ng/ml) of PGE_2 prior to MC activation. Histamine and cytokine release were analyzed in cell supernatants to measure cell activation. As expected, upon MC activation with anti-IgE, all assessed mediators (histamine, TNF-α, IL-13, CCL-2, CCL-3 and IL-6) were increased in cells from both strains (data not shown). However, treatment with PGE_2 prior to MC activation by anti-IgE led to opposing effects, showing a decrease in histamine as well as TNF-α release in BL/6 mice but an increase in Balb/c mice (Fig. 22). There were no differences in the release of other cytokines- IL-6, IL-13, CCL-2 and CCL-3 (data not shown). Similarly, the pre-treatment of BMcMCs with PGE_2 alone did not significantly change the spontaneous

release of histamine or cytokines in either strain (data not shown). This suggests that the suppressive effect of PGE_2 in mice is most likely a result of its impact on MCs.

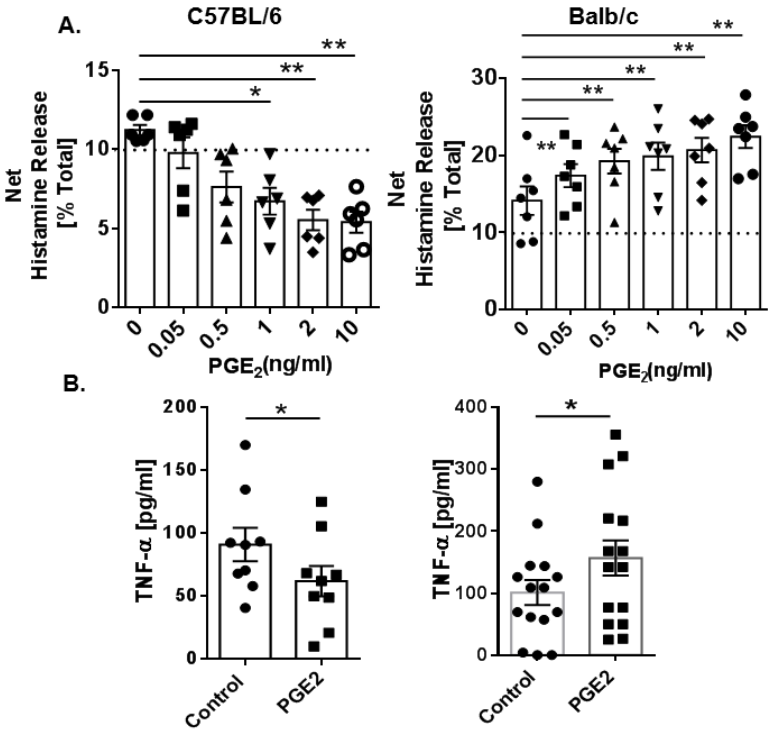

Figure 22: PGE_2 exhibits opposing effects in different strains of mice. BMcMCs from C57BL/6J and Balb/c mice were sensitized and pre-incubated with A) different concentrations of PGE_2 followed by anti-IgE stimulation for 30 min. Histamine release was quantified by an autoanalyzer-based method. The net release was determined in the following way 'Net release= [stimulated release- spontaneous release] in %total'. B) PGE_2 for followed by anti-IgE stimulation for 8h and TNF-α production was measured using ELISA. The data are shown as mean ± SEM, n=5-7 per group. *P< 0.05 and **P<0.01.

6.3.2 EP receptor expression profile is comparable in BL/6- and Balb/c-MCs

The characteristic pattern of expression of PGE_2 receptors, EP 1-4 and their relative ratios in different cells and tissues are known to govern the diverse cellular functions of PGE_2 [151-154]. To assess whether a different EP receptor expression is responsible for the opposing effect of PGE_2 in MCs, we analyzed the relative expression of EP1-4 in BMcMCs from BL/6 and Balb/c mice. Both strains exhibited a comparable expression pattern for EP receptors (Fig.23), with a low expression of EP2 receptor but a strong and comparable expression of EP receptor 3 and 4. Almost no expression

of EP1 receptor was found (data not shown). The expression of EP3 receptor was higher in Balb/c mice compared to BL/6 mice (~1.75 fold).

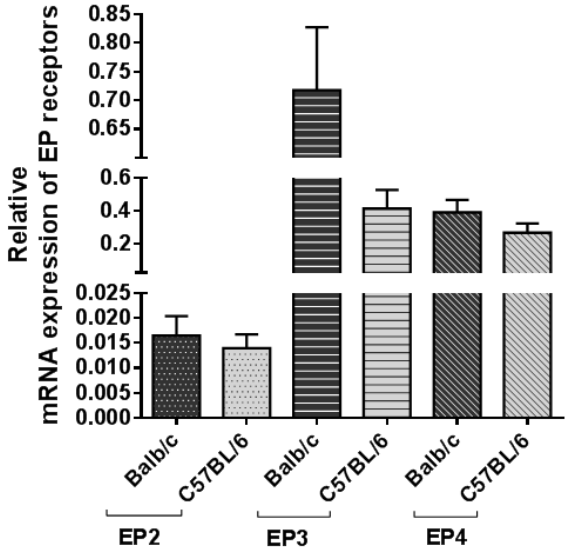

Figure 23: EP receptor expression profile is comparable in BL/6- and Balb/c- MCs. EP receptor expression in BMcMCs was assessed via real time PCR and normalized to the expression of TBP. The data are shown as means ± SEM, n=9.

6.3.3 Impact of PGE_2 in BL/6 mice is mediated by EP2/EP4 receptors

PGE_2 receptors have distinct and partially opposite downstream signaling pathways. Their functional differences are known to impact the effects of PGE_2 [130]. To pinpoint the specific receptor/s responsible for the impact of PGE_2, BMcMCs were treated with specific EP 2-4 agonists, alone or in combination and MCs were stimulated. EP3 agonist stimulated histamine release in MCs from both strains. EP2 and EP4 agonists, however, significantly inhibited histamine release in BMcMCs from BL/6 mice (Fig.24A), but not from Balb/c mice. This effect was even more pronounced upon combination of both agonists (Fig. 24B). In contrast, the combination did not significantly reduce the release of histamine in BMcMCs from Balb/c mice.

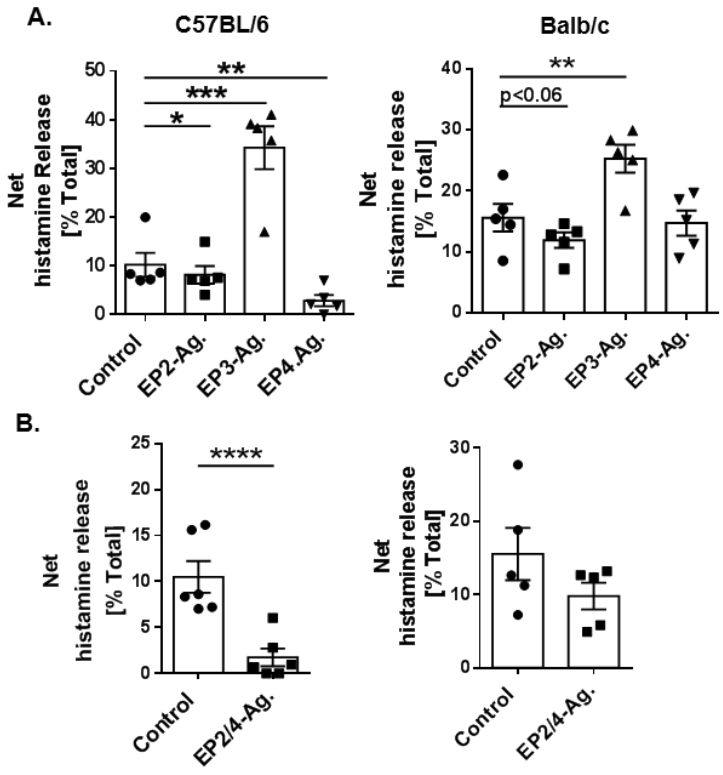

Figure 24: Impact of PGE_2 In C57BL/6J mice is mediated by EP2/EP4 receptors. BMcMCs from C57BL/6 and Balb/c mice were sensitized and pre-incubated with A) different EP agonists. B) EP2 and EP4 agonists combined. MCs were stimulated with anti-IgE and histamine release was quantified by an autoanalyzer-based method. The net release was determined in the following way 'Net release = [stimulated release – spontaneous release] in % of total'. The data are shown as mean ± SEM, n=5-7 per group. *P< 0.05, **P<0.01, ***P<0.001, ****P<0.0001.

6.3.4 Anti-IgE mediated phosphorylation of PLCγ1 and ERK 1/2 is reduced by PGE_2

As PGE_2 impacts IgER-mediated MC activation, we examined the downstream signaling events in MCs to elucidate the mechanism of action of PGE_2. FcεRI mediates signaling by the initial activation of tyrosine kinases like Syk, Lyn and Fyn (Fig. 2) leading to the phosphorylation and activation of downstream enzymes. Degranulation is tightly regulated by two enzymes, PLCγ and PI3K, whereas the MAP kinases ERK1/2, JNK and p38 are involved in cytokine gene transcription (Fig. 2).[16] We verified that FcεRI-aggregation induced phosphorylation of PLCγ1, PI3K, MAP kinase ERK1/2 and p38 in cells from both the strains (Figs. 25/26). To study the impact of PGE_2, BMcMCs were stimulated with PGE_2 alone or together with anti-IgE. The above signaling protein molecules were analyzed regarding phosphorylation in cell lysates.

PGE_2 in combination with anti-IgE inhibited the phosphorylation of PLCγ1 in a dose response manner in BL/6-MCs (Fig. 25) but not in Balb/c-MCs (Fig. 26). The expression of PLCγ2 was low in both strains and was not influenced by PGE_2 (data not shown). ERK1/2 phosphorylation triggered via FcεRI was decreased (Figs. 25/26) in both strains. Conversely, PGE_2 had no effect on p38 (Fig. 25) or JNK (data not shown) in BL/6-MCs, while a trend to an inhibitory activity was identified in Balb/c (Fig. 25). The phosphorylation of AKT, a downstream target and proxy of PI3K activation was found be reduced in Balb/c mice, while less activity was found in cells from BL/6 (Figs. 25/26).

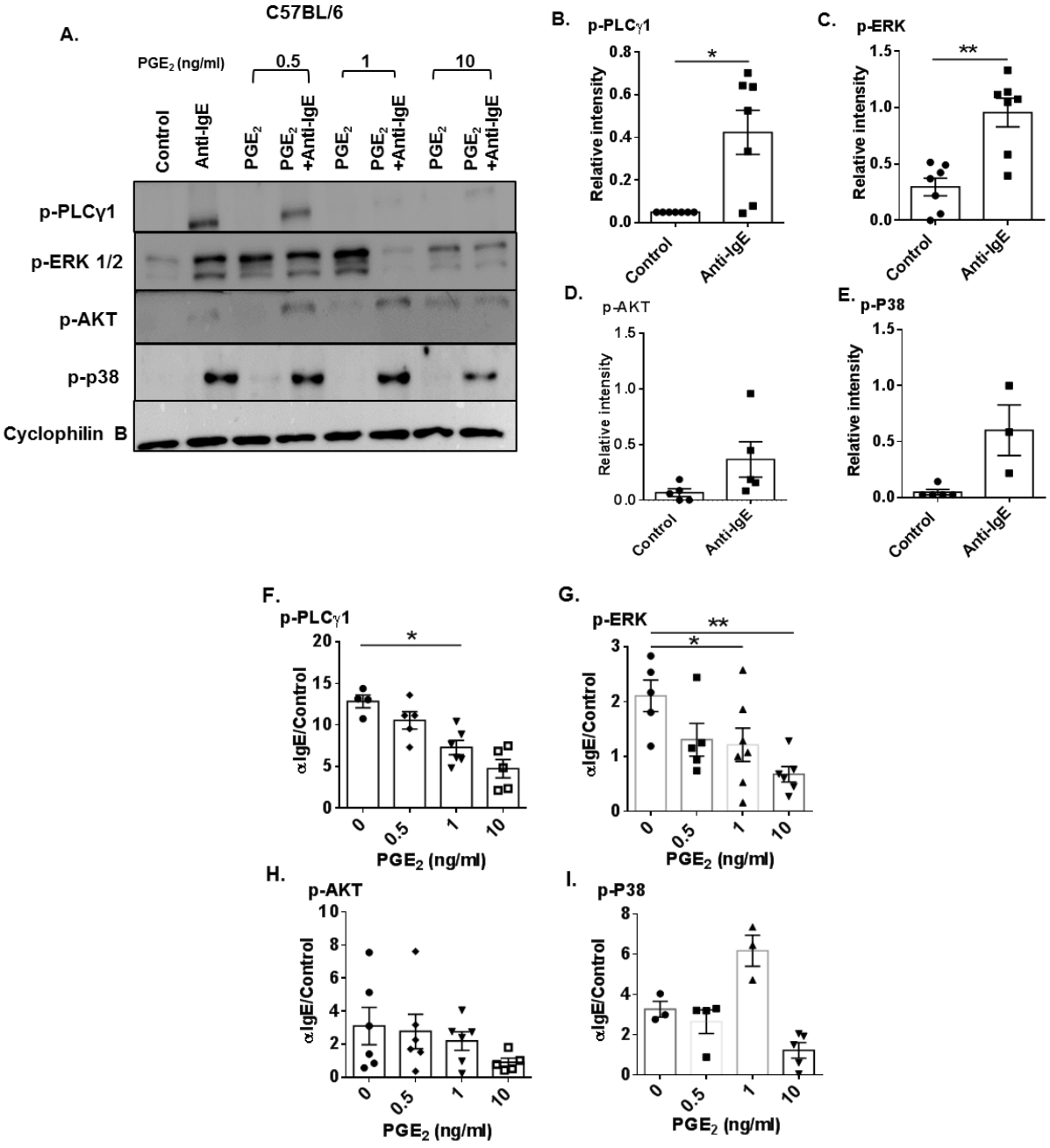

Figure 25: Anti-IgE mediated phosphorylation of PLCγ1 and ERK 1/2 was reduced in C57BL/6J mice. Sensitized BMcMCs were pre-incubated with different concentrations of PGE_2 followed by stimulation with anti-IgE. A) Western blot analysis showing the expression/phosphorylation of mentioned proteins and cyclophilin (as a loading control). B-E) Band intensities obtained by scanning the blots in independent experiments, normalized to cyclophilin B. F-I, Band intensities as in B-E but given as a ratio of stimulated/unstimulated to determine the relative intensities presented under each blot. The data are presented as mean ± SEM, n=5-7 per group. *P< 0.05 and **P<0.01.

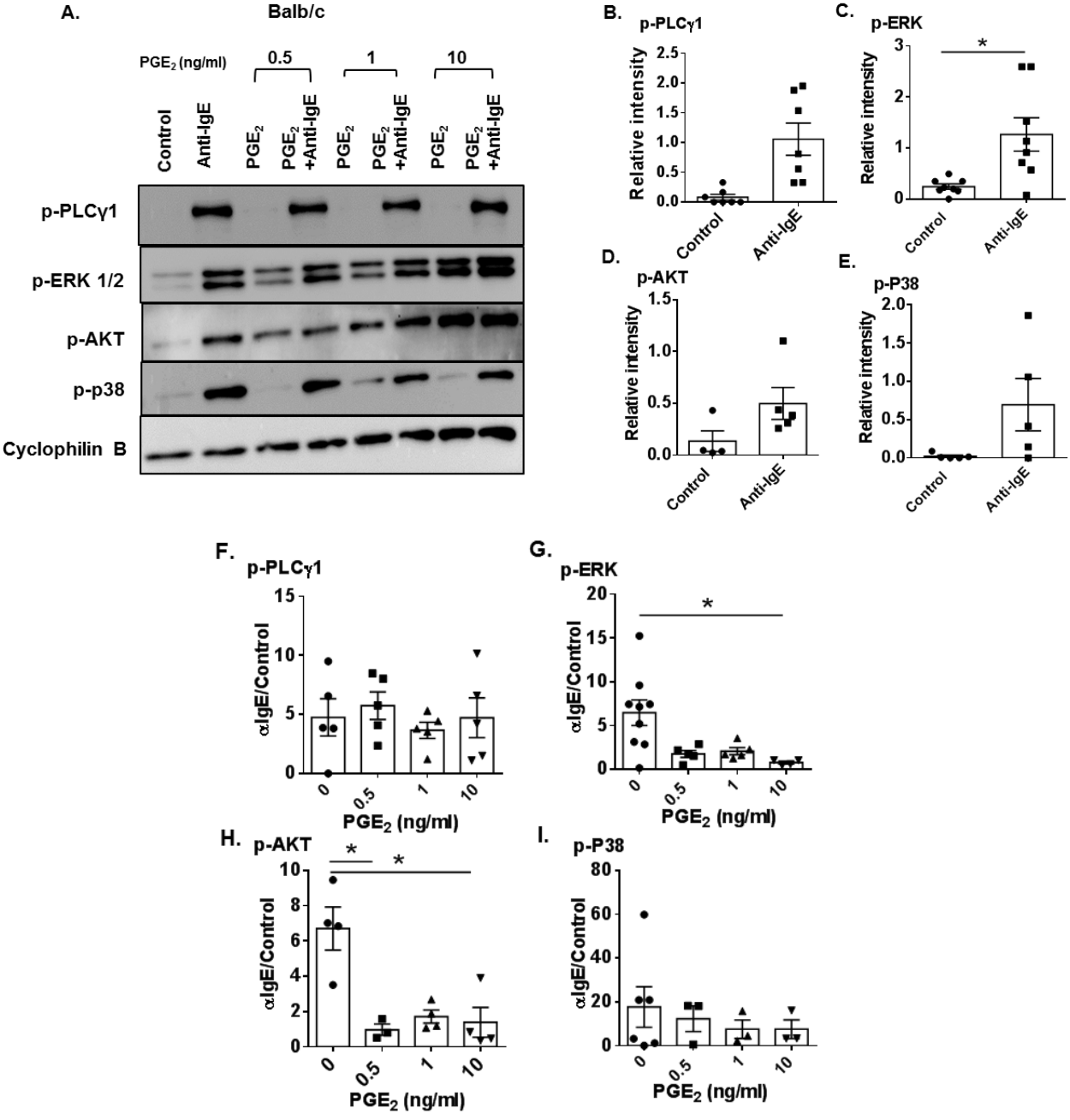

Figure 26: Anti-IgE-mediated phosphorylation of ERK 1/2 and AKT was reduced in Balb/c mice. Sensitized BMcMCs were pre-incubated with different concentrations of PGE_2, followed by stimulation with anti-IgE. A) Western blot analysis showing the expression/phosphorylation of mentioned proteins and cyclophilin (as a loading control). B-E) Band intensities obtained by scanning the blots in independent experiments, normalized to cyclophilin B. F-I, Band intensities as in B-E but given as a ratio of stimulated/unstimulated to determine the relative intensities presented under each blot. The data are presented as mean ± SEM, n=5-7 per group. *P< 0.05.

6.3.5 PGE_2 has a donor-dependent impact on human mast cell activation

PGE_2 displays opposing effects on MC activation in cells from different mouse strains. This raised the question whether this phenomenon can also be replicated in human MCs. We used MCs isolated from breast skin of various donors (HuMCs) and performed histamine release assays. HuMCs were treated with various concentrations of PGE_2, prior to MC activation and histamine release was analyzed in cell supernatants to measure cell activation. Treatment with PGE_2 prior to FcεRI aggregation showed that human MC responses varied across donors both in terms of direction and dose-response pattern replicating findings in the mouse (Fig. 27). These results suggest that the impact of PGE_2 on human MCs and therefore ANA proneness might be influenced by genetic/epigenetic variation.

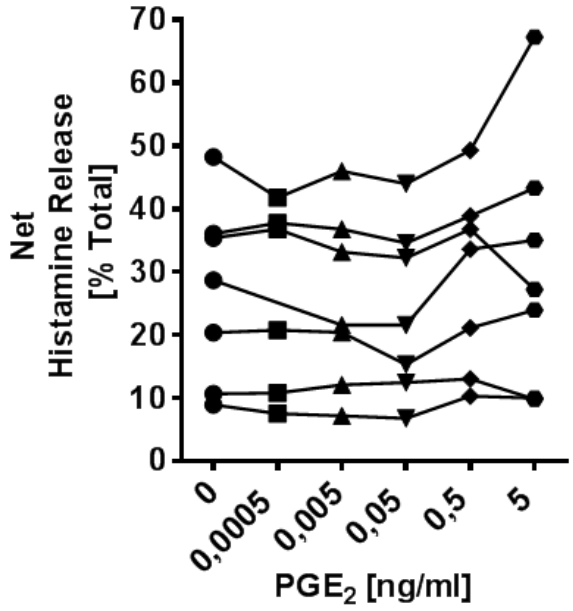

Figure 27: PGE_2 modulates HuMC activation in a donor-dependent fashion. HuMCs were sensitized overnight and pre-incubated with different concentrations of PGE_2. MCs were stimulated and histamine release was quantified by an autoanalyzer-based method. The net release was determined in the following way 'Net release= [stimulated release- spontaneous release] in %total. The data are shown as line graphs where each line corresponds to one donor, n=7.

6.3.6 EP receptor expression profile in mast cells

The impact of PGE_2 in MCs depends on the expression and function of the EP receptors. We analyzed the relative expression of EP receptors 2-4 in HuMCs. HuMCs exhibited prominent expression of EP4, followed by EP3 and EP2 suggesting the significance of EP4 receptor in MCs. These results also mimic the pattern in murine cells (Fig. 28).

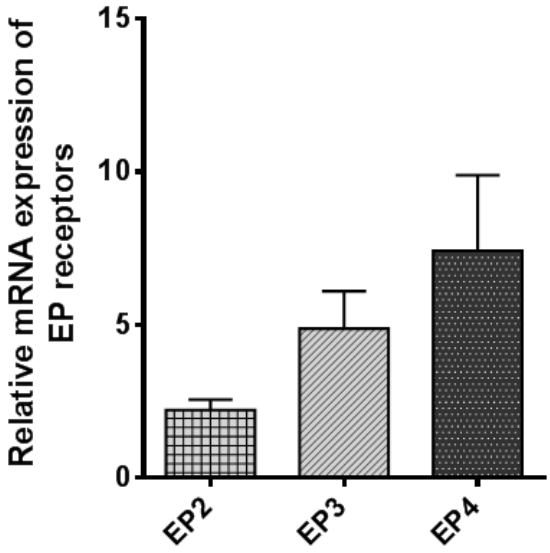

Figure 28: EP receptor expression profile in HuMCs. EP receptor expression in HuMCs was assessed via Real time PCR and normalized to the expression of HPRT. n=6.

6.3.7 Impact of EP agonists on human mast cell activation is donor-dependent

To address the function of EP receptors, MCs were also treated with different doses of EP agonists 2-4, before MC activation was induced. Replacing PGE_2 with EP-selective agonists revealed that the EP2 agonist attenuated MC releasability in a consistent manner (Fig. 29A), while EP3 was stimulatory throughout in the experiments (Fig. 29B). Responsiveness to the EP4 agonist was variable (Fig. 29C), and 2 response patterns were observed: one group displaying elevated and the other group showing decreased degranulation (Fig. 29D). Thus, PGE_2 impacts human skin MC activation in a donor-dependent fashion caused by a variability of EP2/3/4 signal direction and strength across the population, where EP4 seems to be the most relevant receptor.

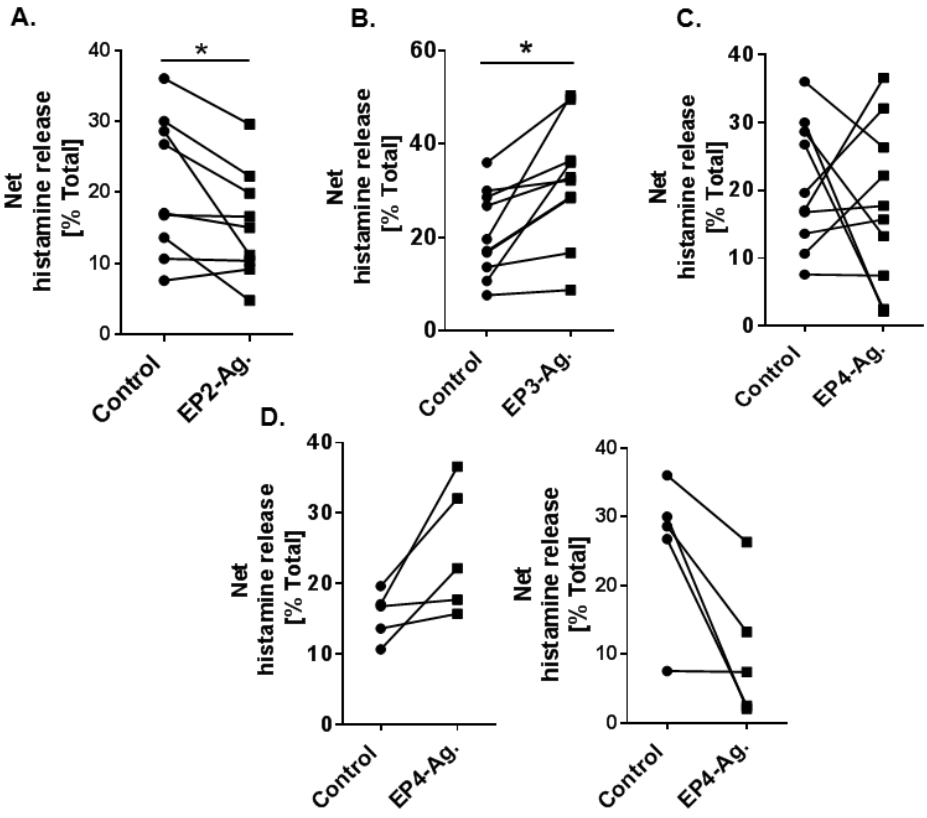

Figure 29: The impact of EP agonists on HuMCs is donor-dependent. HuMCs were sensitized and pre-treated with different EP agonists 2-4. MCs were stimulated with anti-human IgE and histamine release was quantified by an autoanalyzer-based method. The net release was determined in the following way 'Net release= [stimulated release – spontaneous release] in %total. n=9-10. *P< 0.05.

7. DISCUSSION

PGE_2 is an essential homeostatic factor with an emerging role in the regulation of immune functions.[144, 147, 221, 222] Dysregulated PGE_2 has been associated with a wide range of pathological conditions.[223, 224] Studies in the last few decades have reported the aggravation of allergy and hypersensitivity by COX-inhibitors (NSAIDs). [135, 161-166] An imbalance of PGE_2 is believed to be the main cause of NSAID-induced hypersensitivity disorders but it is still not clear. This hypothesis is supported by the clinical benefits of PGE_2 (and synthetic analogues) in patients of airway hyperresponsiveness. [175, 176, 225, 226] Other evidence [173, 174] taken together with data from the European ANA registry [4] also suggest that PGE_2 has a crucial role in allergic responses. The underlying mechanisms of NSAID-mediated hypersensitivity and the deregulations of the PGE_2 system despite some pioneering attempts are still not understood completely.

The aim of this thesis was to better understand the role of human and murine PGE_2 in anaphylactic responses and study the underlying mechanisms. In addition, the influence of genetic variation on the PGE_2 system was examined.

The present data show that PGE_2 deficiency causes susceptibility towards ANA and the pharmacological stabilization of PGE_2 may protect against it. This thesis demonstrates that susceptibility to ANA is caused by MC hyperresponsiveness in the absence of homeostatic levels of PGE_2. Moreover, genetic variation is an important factor influencing the impact of PGE_2 on anaphylactic responses.

7.1 PGE_2 deficiency pre-disposes to anaphylaxis while its stabilization reduces the severity

The intake of COX-inhibitors (NSAIDs) is associated with pro-anaphylactic effects in patients at risk. [124, 167, 173, 227] PGE_2 is a pluripotent prostanoid which has been shown to regulate multiple facets of inflammation and functions of different immune cells such as mast cells. [228] It was hypothesized that a deficiency of PGE_2 resulting from COX-inhibition causes the pre-disposition towards ANA. This hypothesis was supported by studies showing a protective effect of PGE_2 in allergic disease, [156, 167, 216, 229] combined

with differences in PGE_2 abundance in allergic lung disease.[171, 229] Moreover, aggravation of rhinitis or asthma by ASA (COX-inhibitor) is attributed to a lack of PGE_2 secondary to COX-mediated reduction. [159, 170, 172, 230]

Patients with a history of ANA were compared to healthy subjects regarding the levels of systemic PGE_2. It was identified that ANA patients display reduced levels of serum PGE_2 compared to healthy subjects. This finding was consistent with Higashi *et al*. who showed lower levels of baseline PGE_2 in aspirin-intolerant asthma (AIA) patients as compared to the aspirin-tolerant asthma (ATA) group.[171] In addition, nasal polyps and airway fibroblasts isolated from patients of AIA were shown to have impaired production of PGE_2. [170, 172, 216] Stratification of patients by severity grades, showed a negative correlation between serum PGE_2 levels and the severity of anaphylactic reaction within the patient group. However, other biomolecules and MC mediators (such as histamine, tryptase, Cys-LTs and 9α,11β-PGF_2) measured on the same serum samples in connection with our report on biomarker exploration to diagnose ANA, yielded no or minor differences only. [231] These findings together with our data indicate that PGE_2 is reduced in individuals at risk to develop ANA and lack of homeostatic PGE_2 might cause susceptibility towards ANA.

In support of the clinical observations, a multitude of animal models of allergic diseases have shown a protective role of PGE_2 in the elicitation of allergy. [135, 164-166] To confirm the hyposthesis, two mouse strains frequently used in allergy research (Balb/c and BL/6), were compared regarding their basal serum PGE_2 levels and severity of ANA. BL/6 mice developed more severe ANA and possessed significantly lower PGE_2 levels as compared to Balb/c. As in humans, PGE_2 levels were inversely related to ANA severity between the two strains. This data is consistent with previous reports which showed that mice lacking PGE_2 formation due to COX-inhibition or lack of the synthase enzyme gene display increased allergic inflammation. [134, 178]

The presented data strongly indicates that the reduced levels of PGE_2 might cause greater severity of ANA. To further confirm these results, stabilization of PGE_2 was tested and the severity of ANA induced thereafter was analyzed. The rate of PGE_2 degradation is controlled by 15-PGDH enzyme which is responsible for converting it to inactive 15-keto-PGE_2. [228, 232-234] Treatment with a selective 15-PGDH inhibitor (15-PGDH-I), SW033291 in the PSA model increased systemic PGE_2 and also showed a reduction in the severity of ANA in BL/6 mice. The use of SW033291 has been shown to significantly increase PGE_2 levels in mice.[235] Consistent with these results,

immunologically naïve mice lacking 15-PGDH, showed reduced bronchoconstriction in response to methacholine.[236] Similarly, mice with increased PGE_2 levels due to overexpression of PGE_2 synthase in the lung showed reduced airway constriction. [236] This finding confirms that the reduced PGE_2 levels are responsible for a greater anaphylactic severity. The pharmacological stabilization of PGE_2 may be a novel approach to protect against ANA but is however determined by the given genetic background (section 7.3).

7.2 Acetyl salicylic acid aggravates anaphylaxis while PGE_2 protects against it

A strong link between COX-inhibition and the onset of allergic symptoms comes from data about ASA-induced hypersensitivity upon the uptake of ASA/NSAIDs.[161-163] To further confirm the protective effect of PGE_2, ASA-induced aggravation of ANA was studied using a PSA mouse model. *In vivo* treatment with ASA confirmed the exacerbation of ANA replicating the clinical situation in affected patients. However, provision of exogenous PGE_2 completely protected against the pro-anaphylactic effect of ASA. This finding is consistent with the study showing that PGE_2-deficient mice developed ASA sensitivity after induction of allergic airway inflammation.[237] Exogenous PGE_2 is also known to inhibit ASA-induced bronchoconstriction in humans sensitive to these challenges [225, 226] but no such direct studies are reported in mouse models.

Importantly, not only exogenous PGE_2 was able to reduce ANA severity, the stabilization of endogenous PGE_2 by means of 15-PGDH-I was likewise effective at attenuating ANA severity. These results are in agreement with Hartney *et. al* who showed a reduction of airway responsiveness in 15-PGDH knock out mice (Hpgd -/-) and PGE_2 synthase overexpressed mice with increased PGE_2 levels.[236] Lesser potency of the endogenous route can be explained by the lower amount of PGE_2 available for stabilization (especially after COX inhibition). The two approaches together, validate that the deficiency of the prostanoid underlies ANA aggravation after ASA intake.

Based on the literature, COX-1 is the dominant enzyme providing protection from overt allergic responses. [134, 136, 238] The mechanism is also likely in the PSA model of ANA, which is induced in the absence of inflammation or infection. *In vivo,* COX-1 and COX-

2 inhibitors were both able to exacerbate the anaphylactic reaction over basal level, thereby mimicking ASA. These results confirm that the ANA-promoting effect of ASA results from the inhibition of COX activity.[239-242] While SC-560 (COX-1 inhibitor) is reasonably selective (IC50 9 nM for COX-1 and 6,300 nM for COX-2), celecoxib (COX-2 inhibitor) is less selective for its primary target (IC50 40 nM for COX-2 and 1,500 nM COX-1). The results suggest that the COX-2 inhibitor used is insufficient to differentiate between the functions of the two isoforms. Maybe, a more specific inhibitor such as valdecoxib (IC50 5 nM for COX-2 and 140 µM for COX-1) is more suitable due to its high COX-2 specificity. Another approach would be the use of COX-knockout mice which are already under generation for future studies.

PGE_2 acts through EP receptors 1-4, where EP2, EP3 and EP4 are the principal entities due to their broad expression in the body [214, 215] and their potential to exhibit distinct actions in the context of allergy and asthma. [156, 216] Treatment with selective EP agonists [166, 217-220] in the PSA model, showed that all three prostanoid receptors, EP2, EP3 and EP4 were able to reverse ANA-aggravation by ASA further emphasizing the significance of lacking PGE_2 for increased ANA severity. EP2 agonist was more potent, possibly due to its efficient functional coupling to the cAMP pathway despite its lower expression. [216] In line with this finding, EP2 receptor agonist was shown to block airway resistance caused by lysine-aspirin treatment in mice.[237] Similarly, Zaslona *et. al* showed exaggerated airway inflammation in EP2 knock out mice subjected to OVA sensitization and acute airway challenge.[155] Exogenous EP2 stimulation has also been shown to prevent airway hyperresponsiveness and inflammation in mice.[243] Herrerias *et. al* showed EP2 to be overexpressed in house dust mite (HDM)-sensitized mice. This may reflect a compensatory strategy to counteract disease progression. [244] Moreover, in line with our results, EP4 agonist was reported to reduce OVA-induced airway inflammation in a mouse model. [245]

While the results with EP2 and EP4 agonists are expected due to their direct protective effect on MCs in our *in vitro* study, EP3 agonist showed unexpected results since it has a potentiating effect on MCs. Even though EP3 has been viewed as an aggravator of allergy, Kunikata *et al.*[166] and Ueta *et al.*[246] reported that EP3 knockout mice developed more susceptibility to disease in experimental models of allergic asthma and conjunctivitis. Since the effect of EP3 could not be explained through direct MC attenuating function, EP knockout mice will be used in the future to verify these results and delineate the underlying mechanism.

Overall, these data show that the deficiency of PGE_2 in both humans and mice causes a pre-disposition to ANA or aggravation of ANA by COX-inhibitors/ASA. PGE_2 acts via EP receptors 2-4 to reduce anaphylactic responses and maintain its homeostatic function. Moreover, severe ANA could be protected by pharmacological stabilization of PGE_2.

7.3 Genetic variation influences the impact of PGE_2 on anaphylactic responses

Strain dependence in the proneness to develop ANA is known.[62] Reports show differences in the anaphylactic responses of different strains of mice as disease models. [247-250] To study if differential ANA sensitivities result from genetic differences in the PGE_2 network, two mouse strains, Balb/c and BL/6, typically employed in studies of allergic diseases were compared. These mice showed variations in their baseline levels of PGE_2 and severity of systemic ANA. BL/6 mice displayed significantly lower serum PGE_2 levels and developed a profoundly intensified ANA in contrast to their Balb/c counterparts. In line with these findings, Collins *et. al* showed that BL/6 mice developed stronger inflammatory response in comparison to Balb/c mice upon airway challenge. [251] These findings indicate that the PGE_2 system and hence ANA severity is influenced by genetic variation at least when comparing these two strains.

To increase the systemic availability of PGE_2, a stabilizing 15-PGDH inhibitor was administered in both mouse strains. Treatment with the inhibitor increased serum levels of basal PGE_2 in BL/6 mice but had no influence in Balb/c mice. This indicates that the activity or expression of 15-PGDH might be low in Balb/c mice and not responsive to inhibition. The suppression of 15-PGDH activity is reported in many forms of cancer to maintain high levels of PGE_2.[252-255] Since Balb/c mice display tremendous amounts of PGE_2, suppression of enzyme activity might be a possible reason. Genetic polymorphisms which could lead to differential expression and genetic variability are known in the human 15-PGDH enzyme gene [256] and might also be a possibility in Balb/c mice. However, this would need confirmation from further studies but hints at possibly different PGE_2 metabolism in Balb/c mice.

Increase in PGE_2 levels by the inhibitor in BL/6 mice, reduced the severity of ANA and led to quicker recovery. In contrast, the effect of 15-PGDH-I in Balb/c mice could not

be determined. Strain dependent differences in the protective effect of a PGE_2 receptor agonist are reported in mice.[164, 166] These results indicate a possible heterogeneity in the responsiveness of these strains towards PGE_2. However, more animals are required to confirm these results.

These data imply that results obtained for a single genetic background must be viewed critically and extrapolation to the entire population would be inadequate. Overall, these findings highlight that genetic factor may be an essential variable in the PGE_2-MC-ANA connection and explain the conflicting reports about PGE_2's function in the literature.

7.4 PGE_2 protects against anaphylaxis by its direct impact on mast cells

Mast cell activation is a central event in allergic inflammatory responses. MCs are potential targets for immunoregulation by PGE_2.[153] PGE_2 has emerged as an important mediator in studies of MC-related diseases. However, there is a lot of complexity/uncertainty associated with its role.[139, 257] Having found that PGE_2 inhibits the aggravation of ANA in BL/6 mice, the impact of PGE_2 on MCs from both strains was compared. PGE_2 treatment induced opposing effects on anti-IgE mediated histamine release in MCs from both strains. While it increased MC stimulability in Balb/c, PGE_2 reduced MC responsiveness in BL/6 mice. This contrast was also mirrored by a MC cytokine- TNF-α response. TNF-α release was inhibited in BL/6 mice but enhanced in Balb/c by PGE_2 treatment. In the literature, PGE_2 has shown variable and sometimes opposite effects on FcεRI-dependent MC degranulation. It has been shown to decrease the release of mediators like histamine, TNF-α and eicosanoids in rat peritoneal cells,[183] human MCs derived from cord blood[152], peripheral blood progenitors,[187, 190] or human lung MCs.[154] On the contrary, other studies described PGE_2 to exert a stimulatory effect on MC activation.[187-190] It is very important to note though, that the concentrations of PGE_2 used in this study are much higher than in the literature to be consistent with the basal PGE_2 concentrations measured in our mice. There were no differences however, between the two strains regarding other cytokines such as IL-6, IL-13, CCL-2 and CCL-3 (data not shown).

Reasons for this dichotomy are complex. PGE_2 can elicit different responses in MCs from different species,[151-153] different sites,[151, 152, 154] or even different donors.[189]

Increasing evidence [151-154, 188] links this paradox to the existence of four PGE_2 receptors, which are members of the GPCR family with partially opposite downstream pathways. [130, 143, 258] Their characteristic pattern of expression as well as their relative ratios vary across different cells and may explain the diverse PGE_2 functions.[151-154, 259] Analysis of the relative expression of the PGE_2 receptors in BMcMCs revealed a comparably low expression of EP2 receptor and dominant expression of EP3 and EP4 in both strains. However, EP3 receptor was significantly higher expressed in Balb/c mice. EP receptor ratios were revealed as one reason of variation behind the effect of PGE_2,[187] however, the focus was only on the relative expression of EP2 to EP3 on MCs. EP4 was shown to express at a low level and seemed to play a minor role due to its low presence. [260] This data presents evidence demonstrating the importance of the EP4 receptor. Brown *et. al* reported that EP4 is of biological significance only when EP2 is scarcely expressed in a given population.[191]

To determine the specific EP receptor/s contributing to the impact of PGE_2 on MCs, BMcMCs from both strains were treated with EP agonists alone and in combination. As expected, EP3 agonist increases histamine release in both strains. EP3 receptor has been reported to mediate potentiating effects of PGE_2 on the degranulation and cytokine production of murine BMcMCs.[153, 188, 261] The higher expression of EP3 receptor in MCs from Balb/c mice and the strong stimulatory effect of EP3 agonist explain the increasing impact of PGE_2 in Balb/c-MCs.

However, the activation of EP2 and EP4 receptor attenuated histamine release in BL/6-MCs. In particular, EP4 was only effective at reducing MC activation in BL/6 MCs, but not in Balb/c-MCs. The suppressive effect of EP2 and EP4 agonists was more pronounced upon combination in MCs from BL/6. The combination reduced the response by 83% in BL/6 compared to only 36% in Balb/c thereby explaining the inhibition of MC response in BL/6-MCs by PGE_2. In line with these results, a synergistic action of EP2/4 receptors has earlier been reported in other systems such as human aortic smooth muscle cells, where histamine responses were reduced by the activation of EP2 and EP4 receptors and in mouse airway smooth muscle, where cholinergic contraction was similarly reduced by both receptors.[262, 263] The involvement of the two receptors is evident by shared signaling pathways. EP2 and EP4 are both Gs-coupled receptors and act by increasing intracellular cAMP formation, although they also act through their individual alternative pathways.[148] Additionally, EP2 receptor has been

shown to suppress FcεRI-dependent MC degranulation[187] and prevent airway hyperresponsiveness.[164, 243] Similar findings are reported for EP4 receptors.[264-266]

Although, BMcMCs from BL/6 mice show high expression of EP3 receptor and a potentiating effect of the EP3 agonist, the combined inhibitory impact of the EP2 and 4 receptors dominates the overall effect of PGE_2. This may also be reasoned by the highest affinity of EP4 receptor for PGE_2 among the EP receptors.[51] Overall, these results demonstrate that PGE_2 can have diverse effects not only *across* MC subsets [187], but even within the same MC category, governed by genetic variation. Since the receptor expression was largely comparable between the strains, this data suggests a genetic influence on the signaling pathway employed by PGE_2 in the two strains.

IgER (IgE receptor) aggregation on MCs leads to a cascade of tyrosine kinase-initiated events that ultimately lead to the degranulation of MCs by direct or PI3K-mediated PLCγ activation. [62, 73] To investigate the opposing impact of PGE_2 on the release of MC mediators (histamine and cytokines), key signaling events involved in the IgER-mediated degranulation pathway were examined in both mouse strains. As expected, IgER cross-linking led to an increase in the phosphorylation of PLCγ1 and AKT proteins in both Balb/c and BL/6. However, PGE_2 dose-dependently reduced IgER-mediated phosphorylation of PLCγ1 and PI3K (assessed by the phosphorylation of AKT, a surrogate marker for the activation of PI3K) in BL/6-MCs. The expression of PLCγ2, however, was found to be low and the phosphorylation was not detectable although both isoforms have been detected in MCs in the past.[267] PI3K has been shown to have a role in PLCγ activation leading to Ca^{+2} mobilization and degranulation [268]. In line with these results, PGE_2 treatment reduced PLCγ phosphorylation in alveolar macrophages infected with Histoplasma.[269] These findings indicate that the PGE_2-mediated attenuation of MC degranulation in BL/6-MCs takes place through suppression of PLCγ1 and AKT phosphorylation. However, the role of PI3K in PLCγ phosphorylation should be confirmed by using PI3K inhibitors in the future.

Cytokine gene transcription is regulated by the activation of mitogen activated protein kinases (MAPKs; ERKs, c-Jun N-terminal kinases (JNKs), and p38 MAPKs), in MCs. [270, 271] MAPK pathways co-ordinate with each other to regulate TNF-α expression. [272] IgER-mediated phosphorylation of ERK 1/2 likewise, was reduced by PGE_2 in BL/6-MCs potentially explaining the reduction in TNF-α production. Inhibition of ERK activation was reported to impair TNF-α production in monocytes.[273] Despite the trend

to increase basal ERK phosphorylation in PGE_2 treated cells the effect was not significant at any concentration (data not shown). In contrast to ERK 1/2, no impact of PGE_2 on the IgER mediated phosphorylation of p38 and JNK (data not shown) was found. This data is in accordance with studies showing that PGE_2 can prevent the activation of NF-κB and MAP kinases, thereby inhibiting the transcription of pro-inflammatory cytokines.[266, 274] These data indicate that ERK ½ is the only MAPK affected by PGE_2 to downregulate TNF-α production in BL/6-MCs.

However, in Balb/c-MCs, PGE_2 suppresses the phosphorylation of AKT and ERK 1/2 but has no impact on PLCγ1 phosphorylation. These findings indicate the involvement of other players in the potentiation of degranulation and TNF-α release in Balb/c. In agreement with these results, PGE_2 was shown to impair calcium responses, ERK and AKT phosphorylation through activation of EP3 receptors in rat astrocytes.[275] Data from the EP agonist and MC activation experiments indicate the possible role of EP3 receptor in Balb/c-MCs. EP3 receptor was reported to potentiate IgER-mediated degranulation and cytokine production by utilizing a PI3K-independent integration pathway in MCs. This pathway involves the synergistic activation of PLCγ and β. [276] Therefore, in order to find the mechanism underlying the enhancement of degranulation in Balb/c-MCs, the involvement of PLCβ or calcium mobilization should be studied in the future.

With insights of opposing responses to PGE_2 in MCs from different mice strains, we asked whether genetic variation also influences PGE_2 responses in HuMCs. The impact of PGE_2 in HuMCs varied across human donors both in terms of direction and dose-response. To find out if the relative EP receptor 2-4 expression varied among donors, an analysis of the EP receptor expression was performed. Various donors showed a consistent pattern of EP receptor expression with high expression of the EP4 receptor. Moreover, treatment with selective EP agonists showed a consistent reduction in MC release by EP2 agonist throughout the population, while EP3 agonist overall potentiated the release of histamine. Interestingly, donors displayed mixed responses towards the EP4 agonist. High expression of the EP4 receptor and varied MC responses towards EP4 agonist among donors suggest a potential role of this receptor. These data also suggest a protective effect of the EP2 receptor, but its expression is low in these MCs. In the literature, EP4 receptor is known to reduce inflammatory and allergic responses.[277-279] A report published by Gilfillan *et al*. [189] showed that different PGE_2-responder and non-responder populations exhibit

heterogeneity in their responsiveness to PGE_2 due to differences on a molecular level. Therefore, studies involving the EP4 gene or molecular players like PLCγ, ERK 1/2 should be performed to pinpoint differences in the PGE_2 system between donors.

Collectively, these data suggest that the impact of PGE_2 on ANA results from its direct impact on MC activity through the EP receptors. Our direct comparison of mouse strains and human donors reveal the importance of EP2 and EP4 receptors as opposed to only EP2 receptor in the literature. Clearly, further studies using EP knockout mice would help to delve deeper into the role of the three EP receptors and their downstream components in the impact of PGE_2 on MCs and therefore ANA.

7.5 Conclusion and Outlook

This thesis investigated whether and how an impaired PGE_2 system is associated with ANA susceptibility and influenced by genetic background of the host.

The results presented provide new insights into the interplay between COX-PGE_2-MCs-ANA.The data shown revealed that ANA patients and ANA-prone C57BL/6 mice are characterized by reduced levels of PGE_2. Since PGE_2 levels correlated inversely with the severity of anaphylactic reaction, these results indicate that a lack of PGE_2 contributes to ANA susceptibility. Moreover, differential PGE_2 levels between mouse genotypes contribute to differential ANA-susceptibility. BL/6 mice showed better sensitivity towards allergen-induced ANA than Balb/c mice and would prove to be a better model for future studies of ANA. The molecular mechanisms for the lack of PGE_2 in ANA patients are incompletely understood and will be studied on the genomic level.

Stabilization of PGE_2 by inhibiting its degradation or administration of exogenous PGE_2 protects BL/6 mice against ANA and the exacerbation of ANA by ASA. These results confirm that the relative lack of PGE_2 increases the risk or severity of ANA. PGE_2 stabilization may be used to develop a possible therapeutic approach for the management of ANA. However, stabilization of PGE_2 could not be achieved in Balb/c mice indicating possible differences in PGE_2 regulation between the two strains. To get a better understanding of the PGE_2 system in Balb/c mice, the activity and expression of the 15-PGDH enzyme should be studied in the future.

To confirm the protective role of PGE_2 *in vivo*, aggravation of ANA by ASA was studied. ASA worsens ANA through COX-1 and COX-2 inhibition validating the importance of

COX. PGE_2 protects mice against the pro-anaphylactic effect of ASA through its EP receptors 2-4. However, to identify the specific enzyme subset responsible for the protective effects of COX during elicitation of ANA, a more selective inhibitor of COX-2 (such as valdecoxib), in addition to COX-1 and COX-2-deficient mice will be used in the future.

To delineate the mechanism of PGE_2-mediated protection from ANA, the impact of PGE_2 on MC responsiveness was studied. MCs from Balb/c and BL/6 mice exhibit heterogeneity in their responsiveness towards PGE_2 signifying the importance of genetic background. Using selective EP agonists, it was observed that exogenous PGE_2 reduces MC stimulability in BL/6 mice through EP2 and EP4 receptors. However, in Balb/c mice, MC responses are potentiated through the EP3 receptor. PGE_2 develops its protective effect through EP receptors 2 and 4 by direct impact on MCs and by EP3 receptor (*in vivo*) by a mechanism located upstream of MC degranulation. Collectively, all three EP receptors are involved in ANA responses and act to prevent ANA even though the EP3 receptors act in an opposite way on MCs. These results confirm that homeostatic levels of PGE_2 attenuate MC responses through EP receptors 2-4 and reduce ANA susceptibility. The EP3 receptor and the protective impact of the EP receptors in ANA will be studied in more detail using EP knockout mice in the future.

To better understand the heterogeneity in MC responses towards PGE_2 in the two genotypes, MC signaling cascade was studied. PGE_2 reduces MC responses in BL/6 mice by interfering with the phosphorylation of key signaling molecules- PLCγ1 and ERK 1/2. However, the molecular players involved in PGE_2-mediated increase of MC responses in Balb/c could not be determined. Since the data highlights a possible role of the EP3 receptor, signaling intermediates downstream EP3 receptor such as PLCβ or calcium mobilization should be studied in Balb/c mice in the future.

To study if PGE_2 has a protective impact on human MCs, cells from various donors were treated with exogenous PGE_2.The impact of PGE_2 on MC activation varies across donors and was supported by variability in responses towards EP 2/3/4 agonists most importantly EP4. Moreover, HuMCs show dominant expression of the EP4 receptor. Together, these results underline the influence of genetic variation on the PGE_2 system and MC responses. The significance of individual EP receptors in the human PGE_2 network will be studied in detail by knockdown.

The PGE_2 system is complex with diversity at distinct levels comprising PGE_2 synthesis, transport, degradation and function (by differential expression or coupling of EP receptors). Genetic studies of the PGE_2 system in humans and mice and studies using EP knock out mice or EP knockdown in human MCs can be used to gain deeper insights into the intricate PGE_2 network.

To conclude, this thesis reveals an important role of homeostatic PGE_2 and its EP receptors 2-4 in the moderation of ANA susceptibility through direct impact on MCs. In addition, it shows genetic background as an important factor influencing the PGE_2 system and ANA susceptibility. It highlights the potential use of PGDH-inhibitors in therapeutic or preventive strategies for ANA management.

REFERENCES

1. Corry DB, Kheradmand F. Induction and regulation of the IgE response. Nature. 1999;402:18.

2. Gell PGH, Coombs RRA. The classification of allergic reactions underlying disease: Blackwell Science; 1963.

3. Son JH, Park SY, Cho YS, Chung BY, Kim HO, Park CW. Immediate Hypersensitivity Reactions Induced by Triamcinolone in a Patient with Atopic Dermatitis. Journal of Korean medical science. 2018;33(12):e87-e.

4. Worm M, Edenharter G, Rueff F, Scherer K, Pfohler C, Mahler V, et al. Symptom profile and risk factors of anaphylaxis in Central Europe. Allergy. 2012;67(5):691-8.

5. Geha RS, Jabara HH, Brodeur SR. The regulation of immunoglobulin E class-switch recombination. Nature Reviews Immunology. 2003;3(9):721-32.

6. Akdis C. GR, Kay B, Nunez G, Renz H, Shaffer A. Allergy and hypersensitivity. Murphy K, Travers P, Walport M, eds Janeways's Immunobiology. New York and London: Garland Science, Taylor & Francis Group, LLC; 2008. p. 555–98.

7. Sutton BJ, Gould HJ. The human IgE network. Nature. 1993;366(6454):421-8.

8. Wu LC, Zarrin AA. The production and regulation of IgE by the immune system. Nature Reviews Immunology. 2014;14:247.

9. Eckl-Dorna J, Niederberger V. What is the source of serum allergen-specific IgE? Current Allergy and Asthma Reports. 2013;13(3):281-7.

10. Galli SJ, Tsai M. IgE and mast cells in allergic disease. Nature medicine. 2012;18(5):693-704.

11. Devereux G. The increase in the prevalence of asthma and allergy: food for thought. Nature Reviews Immunology. 2006;6(11):869-74.

12. Pawankar M, Canonica GW, MD, Holgate ST, Lockey RF. World Health Organization. White Book on Allergy, Executive Summary. 2011-2012.

13. Ishizaka K, Tomioka H, Ishizaka T. Mechanisms of passive sensitization. I. Presence of IgE and IgG molecules on human leukocytes. Journal of Immunology (Baltimore, Md : 1950). 1970;105(6):1459-67.

14. Gould HJ, Sutton BJ. IgE in allergy and asthma today. Nature Reviews Immunology. 2008;8(3):205-17.

15. Platzer B, Ruiter F, Van der Mee J, Fiebiger E. Soluble IgE receptors--elements of the IgE network. Immunology letters. 2011;141(1):36-44.

16. Sonoda T, Kitamura Y, Haku Y, Hara H, Mori KJ. Mast-cell precursors in various haematopoietic colonies of mice produced in vivo and in vitro. Bristish Journal of Haematology. 1983;53(4):611-20.

17. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiological Reviews. 1997;77(4):1033-79.

18. Metzger H. The receptor with high affinity for IgE. Immunological Reviews. 1992;125:37-48.

19. Amin K. The role of mast cells in allergic inflammation. Respiratory Medicine. 2012;106(1):9-14.

20. Galli SJ, Tsai M. Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity. European Journal of Immunology. 2010;40(7):1843-51.

21. Williams CM, Galli SJ. The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. The Journal of Allergy and Clinical Immunology. 2000;105(5):847-59.

22. Kambe N, Hiramatsu H, Shimonaka M, Fujino H, Nishikomori R, Heike T, et al. Development of both human connective tissue-type and mucosal-type mast cells in mice from hematopoietic stem cells with identical distribution pattern to human body. Blood. 2004;103(3):860-7.
23. Kitamura Y, Oboki K, Ito A. Development of mast cells. Proceedings of the Japan Academy Ser B Physical and Biological Sciences. 2007;83(6):164-74.
24. Enerback L. Mast cells in rat gastrointestinal mucosa. I. Effects of fixation. Acta pathologica et microbiologica Scandinavica. 1966;66(3):289-302.
25. Xing W, Austen KF, Gurish MF, Jones TG. Protease phenotype of constitutive connective tissue and of induced mucosal mast cells in mice is regulated by the tissue. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(34):14210-5.
26. Reynolds DS, Stevens RL, Lane WS, Carr MH, Austen KF, Serafin WE. Different mouse mast cell populations express various combinations of at least six distinct mast cell serine proteases. Proceedings of the National Academy of Sciences of the United States of America. 1990;87(8):3230-4.
27. Ekoff M, Strasser A, Nilsson G. FcepsilonRI aggregation promotes survival of connective tissue-like mast cells but not mucosal-like mast cells. Journal of Immunology (Baltimore, Md : 1950). 2007;178(7):4177-83.
28. Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease compositions. Proceedings of the National Academy of Sciences of the United States of America. 1986;83(12):4464-8.
29. Schwartz LB. Analysis of MC_T and MC_{TC} Mast Cells in Tissue. Mast Cells. Methods In Molecular Biology 2005.
30. Irani AM, Schwartz LB. Mast cell heterogeneity. Clinical and Experimental Allergy. 1989;19(2):143-55.
31. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. Nature Immunology. 2005;6(2):135-42.
32. Tkaczyk C, Jensen BM, Iwaki S, Gilfillan AM. Adaptive and innate immune reactions regulating mast cell activation: from receptor-mediated signaling to responses. Immunology and Allergy Clinics of North America. 2006;26(3):427-50.
33. Stassen M, Hultner L, Schmitt E. Classical and alternative pathways of mast cell activation. Critical Reviews in Immunology. 2002;22(2):115-40.
34. Turner H, Kinet JP. Signalling through the high-affinity IgE receptor Fc epsilonRI. Nature. 1999;402(6760 Suppl):B24-30.
35. Chen T, Repetto B, Chizzonite R, Pullar C, Burghardt C, Dharm E, et al. Interaction of phosphorylated FcepsilonRIgamma immunoglobulin receptor tyrosine activation motif-based peptides with dual and single SH2 domains of p72syk. Assessment of binding parameters and real time binding kinetics. The Journal of Biological Chemistry. 1996;271(41):25308-15.
36. Kovarova M, Tolar P, Arudchandran R, Draberova L, Rivera J, Draber P. Structure-function analysis of Lyn kinase association with lipid rafts and initiation of early signaling events after Fcepsilon receptor I aggregation. Molecular and Cellular Biology. 2001;21(24):8318-28.
37. Chelombitko MA, Fedorov AV, Ilyinskaya OP, Zinovkin RA, Chernyak BV. Role of Reactive Oxygen Species in Mast Cell Degranulation. Biochemistry Biokhimiia. 2016;81(12):1564-77.

38. Eiseman, Bolen JB. Engagement of the high-affinity IgE receptor activates src protein-related tyrosine kinases. Nature. 1992;355(6355):78-80.
39. Rivera J, Gilfillan AM. Molecular regulation of mast cell activation. The Journal of Allergy and Clinical Immunology. 2006;117(6):1214-25; quiz 26.
40. Saitoh S, Arudchandran R, Manetz TS, Zhang W, Sommers CL, Love PE, et al. LAT is essential for Fc(epsilon)RI-mediated mast cell activation. Immunity. 2000;12(5):525-35.
41. Rivera J. NTAL/LAB and LAT: a balancing act in mast-cell activation and function. Trends in Immunology. 2005;26(3):119-22.
42. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. Nature Reviews Immunology. 2006;6(3):218-30.
43. Silverman MA, Shoag J, Wu J, Koretzky GA. Disruption of SLP-76 interaction with Gads inhibits dynamic clustering of SLP-76 and FcepsilonRI signaling in mast cells. Molecular and Cellular Biology. 2006;26(5):1826-38.
44. Rhee SG. Regulation of phosphoinositide-specific phospholipase C. Annual Review of Biochemistry. 2001;70:281-312.
45. Beaven MA, Metzger H. Signal transduction by Fc receptors: the Fc epsilon RI case. Immunology Today. 1993;14(5):222-6.
46. Kawakami Y, Kitaura J, Satterthwaite AB, Kato RM, Asai K, Hartman SE, et al. Redundant and opposing functions of two tyrosine kinases, Btk and Lyn, in mast cell activation. Journal of Immunology (Baltimore, Md : 1950). 2000;165(3):1210-9.
47. Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, et al. Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. Nature Immunology. 2002;3(8):741-8.
48. Olivera A, Mizugishi K, Tikhonova A, Ciaccia L, Odom S, Proia RL, et al. The Sphingosine Kinase-Sphingosine-1-Phosphate Axis Is a Determinant of Mast Cell Function and Anaphylaxis. Immunity. 2007;26(3):287-97.
49. Choi OH, Kim JH, Kinet JP. Calcium mobilization via sphingosine kinase in signalling by the Fc epsilon RI antigen receptor. Nature. 1996;380(6575):634-6.
50. Hait NC, Oskeritzian CA, Paugh SW, Milstien S, Spiegel S. Sphingosine kinases, sphingosine 1-phosphate, apoptosis and diseases. Biochimica et Biophysica Acta. 2006;1758(12):2016-26.
51. Lee HS, Park CS, Lee YM, Suk HY, Clemons TC, Choi OH. Antigen-induced Ca2+ mobilization in RBL-2H3 cells: role of I(1,4,5)P3 and S1P and necessity of I(1,4,5)P3 production. Cell Calcium. 2005;38(6):581-92.
52. Bingham CO, Austen KF. Phospholipase A2 enzymes in eicosanoid generation. Proceedings of the Association of American Physicians. 1999;111(6):516-24.
53. Lorentz A, Klopp I, Gebhardt T, Manns MP, Bischoff SC. Role of activator protein 1, nuclear factor-kappaB, and nuclear factor of activated T cells in IgE receptor-mediated cytokine expression in mature human mast cells. The Journal of Allergy and Clinical Immunology. 2003;111(5):1062-8.
54. Kitaura J, Asai K, Maeda-Yamamoto M, Kawakami Y, Kikkawa U, Kawakami T. Akt-dependent cytokine production in mast cells. Journal of Experimental Medicine. 2000;192(5):729-40.

55. Kushnir-Sukhov NM, Brown JM, Wu Y, Kirshenbaum A, Metcalfe DD. Human mast cells are capable of serotonin synthesis and release. The Journal of Allergy and Clinical Immunology. 2007;119(2):498-9.
56. Metcalfe DD. Mast cells and mastocytosis. Blood. 2008;112(4):946-56.
57. Bischoff SC. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. Nature Reviews Immunology. 2007;7(2):93-104.
58. Finkelman FD. Anaphylaxis: lessons from mouse models. The Journal of Allergy and Clinical Immunology. 2007;120(3):506-15; quiz 16-7.
59. Wang J, Sampson HA. Food anaphylaxis. Clinical and Experimental Allergy. 2007;37(5):651-60.
60. Hata AN, Breyer RM. Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacology and Therapeutics. 2004;103(2):147-66.
61. Pejler G AM, Ringvall M, Wernersson S. Advances in Immunology: Elsevier Inc.; 2007. 167-77 p.
62. Metcalfe DD, Peavy RD, Gilfillan AM. Mechanisms of mast cell signaling in anaphylaxis. The Journal of Allergy and Clinical Immunology. 2009;124(4):639-46; quiz 47-8.
63. Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF, Jr., Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report--Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. The Journal of Allergy and Clinical Immunology. 2006;117(2):391-7.
64. Dhami S, Panesar SS, Roberts G, Muraro A, Worm M, Bilo MB, et al. Management of anaphylaxis: a systematic review. Allergy. 2014;69(2):168-75.
65. Johansson SG, Bieber T, Dahl R, Friedmann PS, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. The Journal of Allergy and Clinical Immunology. 2004;113(5):832-6.
66. Ring J, Grosber M, Brockow K, Bergmann KC. Anaphylaxis. Chemical Immunology and Allergy. 2014;100:54-61.
67. Dale HH, Laidlaw PP. Histamine shock. The Journal of Physiology. 1919;52(5):355-90.
68. Hanzlik PJ, Karsner HT. Anaphylactoid Phenomena from the intravenous administration of various colloids, arsenicals and other agents. Journal of Pharmacology and Experimental Therapeutics. 1920;14(5):379-423.
69. Mueller HL. Diagnosis and treatment of insect sensitivity. The Journal of Asthma Research. 1966;3(4):331-3.
70. Ring J, Messmer K. Incidence and severity of anaphylactoid reactions to colloid volume substitutes. Lancet. 1977;1(8009):466-9.
71. Oettgen HC. Fifty years later: Emerging functions of IgE antibodies in host defense, immune regulation, and allergic diseases. The Journal of Allergy and Clinical Immunology. 2016;137(6):1631-45.
72. Platts-Mills TAE, Schuyler AJ, Erwin EA, Commins SP, Woodfolk JA. IgE in the diagnosis and treatment of allergic disease. The Journal of Allergy and Clinical Immunology. 2016;137(6):1662-70.
73. Kraft S, Kinet JP. New developments in FcepsilonRI regulation, function and inhibition. Nature Reviews Immunology. 2007;7(5):365-78.

74. Wershil BK, Mekori YA, Murakami T, Galli SJ. 125I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells. The Journal of Immunology (Baltimore, Md : 1950). 1987;139(8):2605-14.
75. Dombrowicz D, Flamand V, Brigman KK, Koller BH, Kinet JP. Abolition of anaphylaxis by targeted disruption of the high affinity immunoglobulin E receptor alpha chain gene. Cell. 1993;75(5):969-76.
76. Hirayama N, Hirano T, Kohler G, Kurata A, Okumura K, Ovary Z. Biological activities of antitrinitrophenyl and antidinitrophenyl mouse monoclonal antibodies. Proceedings of the National Academy of Sciences of the United States of America. 1982;79(2):613-5.
77. Feyerabend TB, Weiser A, Tietz A, Stassen M, Harris N, Kopf M, et al. Cre-mediated cell ablation contests mast cell contribution in models of antibody- and T cell-mediated autoimmunity. Immunity. 2011;35(5):832-44.
78. Oka T, Kalesnikoff J, Starkl P, Tsai M, Galli SJ. Evidence questioning cromolyn's effectiveness and selectivity as a 'mast cell stabilizer' in mice. Laboratory Investigation. 2012;92(10):1472-82.
79. Lilla JN, Chen CC, Mukai K, BenBarak MJ, Franco CB, Kalesnikoff J, et al. Reduced mast cell and basophil numbers and function in Cpa3-Cre; Mcl-1fl/fl mice. Blood. 2011;118(26):6930-8.
80. Beutier H, Gillis CM, Iannascoli B, Godon O, England P, Sibilano R, et al. IgG subclasses determine pathways of anaphylaxis in mice. The Journal of Allergy and Clinical Immunology. 2017;139(1):269-80.e7.
81. Jonsson F, Mancardi DA, Kita Y, Karasuyama H, Iannascoli B, Van Rooijen N, et al. Mouse and human neutrophils induce anaphylaxis. Journal of Clinical Investigation. 2011;121(4):1484-96.
82. Tsujimura Y, Obata K, Mukai K, Shindou H, Yoshida M, Nishikado H, et al. Basophils play a pivotal role in immunoglobulin-G-mediated but not immunoglobulin-E-mediated systemic anaphylaxis. Immunity. 2008;28(4):581-9.
83. Khodoun MV, Strait R, Armstrong L, Yanase N, Finkelman FD. Identification of markers that distinguish IgE- from IgG-mediated anaphylaxis. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(30):12413-8.
84. Strait RT, Morris SC, Yang M, Qu XW, Finkelman FD. Pathways of anaphylaxis in the mouse. The Journal of Allergy and Clinical Immunology. 2002;109(4):658-68.
85. Khodoun MV, Kucuk ZY, Strait RT, Krishnamurthy D, Janek K, Clay CD, et al. Rapid desensitization of mice with anti-FcgammaRIIb/FcgammaRIII mAb safely prevents IgG-mediated anaphylaxis. The Journal of Allergy and Clinical Immunology. 2013;132(6):1375-87.
86. Dombrowicz D, Flamand V, Miyajima I, Ravetch JV, Galli SJ, Kinet JP. Absence of Fc epsilonRI alpha chain results in upregulation of Fc gammaRIII-dependent mast cell degranulation and anaphylaxis. Evidence of competition between Fc epsilonRI and Fc gammaRIII for limiting amounts of FcR beta and gamma chains. Journal of Clinical Investigation. 1997;99(5):915-25.
87. Oettgen HC, Martin TR, Wynshaw-Boris A, Deng C, Drazen JM, Leder P. Active anaphylaxis in IgE-deficient mice. Nature. 1994;370(6488):367-70.
88. Nimmerjahn F, Bruhns P, Horiuchi K, Ravetch JV. FcgammaRIV: a novel FcR with distinct IgG subclass specificity. Immunity. 2005;23(1):41-51.

89. Nimmerjahn F, Ravetch JV. Divergent immunoglobulin g subclass activity through selective Fc receptor binding. Science. 2005;310(5753):1510-2.
90. Cheifetz A, Smedley M, Martin S, Reiter M, Leone G, Mayer L, et al. The incidence and management of infusion reactions to infliximab: a large center experience. American Journal of Gastroenterology. 2003;98(6):1315-24.
91. Schmidt AP, Taswell HF, Gleich GJ. Anaphylactic Transfusion Reactions Associated with Anti-IgA Antibody. New England Journal of Medicine. 1969;280(4):188-93.
92. Steenholdt C, Svenson M, Bendtzen K, Thomsen OO, Brynskov J, Ainsworth MA. Acute and delayed hypersensitivity reactions to infliximab and adalimumab in a patient with Crohn's disease. Journal of Crohn's and Colitis. 2012;6(1):108-11.
93. Farnam K, Chang C, Teuber S, Gershwin ME. Nonallergic drug hypersensitivity reactions. International Archives of Allergy and Immunology. 2012;159(4):327-45.
94. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. Journal of Clinical Investigation. 2006;116(3):833-41.
95. Szebeni J. Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity. Toxicology. 2005;216(2-3):106-21.
96. Klos A, Tenner AJ, Johswich KO, Ager RR, Reis ES, Kohl J. The role of the anaphylatoxins in health and disease. Molecular Immunology. 2009;46(14):2753-66.
97. Smith PL, Kagey-Sobotka A, Bleecker ER, Traystman R, Kaplan AP, Gralnick H, et al. Physiologic manifestations of human anaphylaxis. The Journal of Cinical Investigation. 1980;66(5):1072-80.
98. Brown SG, Stone SF, Fatovich DM, Burrows SA, Holdgate A, Celenza A, et al. Anaphylaxis: clinical patterns, mediator release, and severity. The Journal of Allergy and Clinical Immunology. 2013;132(5):1141-9.e5.
99. Lepow IH, Willms-Kretschmer K, Patrick RA, Rosen FS. Gross and ultrastructural observations on lesions produced by intradermal injection of human C3a in man. The American Journal of Pathology. 1970;61(1):13-23.
100. Wuepper KD, Bokisch VA, Muller-Eberhard HJ, Stoughton RB. Cutaneous responses to human C 3 anaphylatoxin in man. Clinical and Experimental Immunology. 1972;11(1):13-20.
101. Yancey KB, Hammer CH, Harvath L, Renfer L, Frank MM, Lawley TJ. Studies of human C5a as a mediator of inflammation in normal human skin. Journal of Clinical Investigation. 1985;75(2):486-95.
102. Gorski JP, Hugli TE, Muller-Eberhard HJ. C4a: the third anaphylatoxin of the human complement system. Proceedings of the National Academy of Sciences of the United States of America. 1979;76(10):5299-302.
103. Schäfer B, Piliponsky AM, Oka T, Song CH, Gerard NP, Gerard C, et al. Mast cell anaphylatoxin receptor expression can enhance IgE-dependent skin inflammation in mice. The Journal of Allergy and Clinical Immunology. 2013;131(2):541-8.e89.
104. Jiao D, Liu Y, Lu X, Liu B, Pan Q, Liu Y, et al. Macrophages are the dominant effector cells responsible for IgG-mediated passive systemic anaphylaxis challenged by natural protein antigen in BALB/c and C57BL/6 mice. Cellular Immunology. 2014;289(1-2):97-105.

105. Windbichler M, Echtenacher B, Takahashi K, Ezekowitz RA, Schwaeble WJ, Jenseniuis JC, et al. Investigations on the involvement of the lectin pathway of complement activation in anaphylaxis. International Archives of Allergy and Immunology. 2006;141(1):11-23.
106. Wolbing F, Fischer J, Koberle M, Kaesler S, Biedermann T. About the role and underlying mechanisms of cofactors in anaphylaxis. Allergy. 2013;68(9):1085-92.
107. Worm M, Babina M, Hompes S. Causes and risk factors for anaphylaxis. Journal der Deutschen Dermatologischen Gesellschaft. 2013;11(1):44-50.
108. Worm M, Francuzik W, Renaudin JM, Bilo MB, Cardona V, Hofmeier KS, et al. Factors increasing the risk for a severe reaction in anaphylaxis: An analysis of data from The European Anaphylaxis Registry. Allergy. 2018;73(6):1322-30.
109. Worm M, Moneret-Vautrin A, Scherer K, Lang R, Fernandez-Rivas M, Cardona V, et al. First European data from the network of severe allergic reactions (NORA). Allergy. 2014;69(10):1397-404.
110. Turner PJ, Baumert JL, Beyer K, Boyle RJ, Chan CH, Clark AT, et al. Can we identify patients at risk of life-threatening allergic reactions to food? Allergy. 2016;71(9):1241-55.
111. Munoz-Cano R, Pascal M, Araujo G, Goikoetxea MJ, Valero AL, Picado C, et al. Mechanisms, Cofactors, and Augmenting Factors Involved in Anaphylaxis. Frontiers in Immunology. 2017;8:1193.
112. Cant AJ, Gibson P, Dancy M. Food hypersensitivity made life threatening by ingestion of aspirin. British Medical Journal (Clinical research ed). 1984;288(6419):755-6.
113. Moneret-Vautrin DA, Latarche C. [Drugs as risk factors of food anaphylaxis in adults: a case-control study]. Bulletin de L'Academie Nationale de Medicine. 2009;193(2):351-62.
114. Cardona V, Luengo O, Garriga T, Labrador-Horrillo M, Sala-Cunill A, Izquierdo A, et al. Co-factor-enhanced food allergy. Allergy. 2012;67(10):1316-8.
115. Pascal M, Munoz-Cano R, Reina Z, Palacin A, Vilella R, Picado C, et al. Lipid transfer protein syndrome: clinical pattern, cofactor effect and profile of molecular sensitization to plant-foods and pollens. Clinical and Experimental Allergy. 2012;42(10):1529-39.
116. Pascal M, Munoz-Cano R, Mila J, Sanz ML, Diaz-Perales A, Sanchez-Lopez J, et al. Nonsteroidal anti-inflammatory drugs enhance IgE-mediated activation of human basophils in patients with food anaphylaxis dependent on and independent of nonsteroidal anti-inflammatory drugs. Clinical and Experimental Allergy. 2016;46(8):1111-9.
117. Wojnar RJ, Hearn T, Starkweather S. Augmentation of allergic histamine release from human leukocytes by nonsteroidal anti-inflammatory-analgesic agents. The Journal of Allergy and Clinical Immunology. 1980;66(1):37-45.
118. Knights KM, Mangoni AA, Miners JO. Defining the COX inhibitor selectivity of NSAIDs: implications for understanding toxicity. Expert Review of Clinical Pharmacology. 2010;3(6):769-76.
119. Kowalski ML, Asero R, Bavbek S, Blanca M, Blanca-Lopez N, Bochenek G, et al. Classification and practical approach to the diagnosis and management of hypersensitivity to nonsteroidal anti-inflammatory drugs. Allergy. 2013;68(10):1219-32.
120. Teodosiev LS. [Importance of prostaglandins in maintaining homeostasis]. Vutreshni bolesti. 1975;14(2):1-12.
121. Lee JB, Patak RV, Mookerjee BK. Renal prostaglandins and the regulation of blood pressure and sodium and water homeostasis. The American Journal of Medicine. 1976;60(6):798-816.

122. Kishimoto TK, Viswanathan K, Ganguly T, Elankumaran S, Smith S, Pelzer K, et al. Contaminated heparin associated with adverse clinical events and activation of the contact system. New England Journal of Medicine. 2008;358(23):2457-67.
123. Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. The Journal of Allergy and Clinical Immunology. 2003;111(5):913-21; quiz 22.
124. Morales DR, Guthrie B, Lipworth BJ, Jackson C, Donnan PT, Santiago VH. NSAID-exacerbated respiratory disease: a meta-analysis evaluating prevalence, mean provocative dose of aspirin and increased asthma morbidity. Allergy. 2015;70(7):828-35.
125. Campbell WB. Lipid-derived autacoids : eicosanoids and platelet-activating factor. Goodman and Gilman's, The Pharmacological Basis of Therapeutics. 1990;600.
126. Kudo I, Murakami M. Prostaglandin E synthase, a terminal enzyme for prostaglandin E2 biosynthesis. Journal of Biochemistry and Molecular Biology. 2005;38(6):633-8.
127. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annual Review of Pharmacology and Toxicology. 1998;38(1):97-120.
128. Simmons DL, Botting RM, Hla T. Cyclooxygenase isozymes: the biology of prostaglandin synthesis and inhibition. Pharmacological Reviews. 2004;56(3):387-437.
129. Chell S, Kadi A, Caroline WA, Paraskeva C. Mediators of PGE2 synthesis and signalling downstream of COX-2 represent potential targets for the prevention/treatment of colorectal cancer. Biochimica et Biophysica Acta - Reviews on Cancer. 2006;1766(1):104-19.
130. Sugimoto Y, Narumiya S. Prostaglandin E receptors. The Journal of Biological Chemistry. 2007;282(16):11613-7.
131. Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. Arteriosclerosis, Thrombosis, and Vascular biology. 2011;31(5):986-1000.
132. Ivanov AI, Romanovsky AA. Prostaglandin E2 as a mediator of fever: synthesis and catabolism. Frontiers in Bioscience. 2004;9:1977-93.
133. Vane JR. Inhibition of Prostaglandin Synthesis as a Mechanism of Action for Aspirin-like Drugs. Nature New Biology. 1971;231(25):232-5.
134. Gavett SH, Madison SL, Chulada PC, Scarborough PE, Qu W, Boyle JE, et al. Allergic lung responses are increased in prostaglandin H synthase-deficient mice. The Journal of Clinical Investigation. 1999;104(6):721-32.
135. Peebles RS, Jr., Dworski R, Collins RD, Jarzecka K, Mitchell DB, Graham BS, et al. Cyclooxygenase inhibition increases interleukin 5 and interleukin 13 production and airway hyperresponsiveness in allergic mice. American Journal of Respiratory and Critical care medicine. 2000;162(2 Pt 1):676-81.
136. Carey MA, Germolec DR, Bradbury JA, Gooch RA, Moorman MP, Flake GP, et al. Accentuated T helper type 2 airway response after allergen challenge in cyclooxygenase-1-/- but not cyclooxygenase-2-/- mice. American Journal of Respiratory and Critical care medicine. 2003;167(11):1509-15.
137. Nakata J, Kondo M, Tamaoki J, Takemiya T, Nohara M, Yamagata K, et al. Augmentation of allergic inflammation in the airways of cyclooxygenase-2-deficient mice. Respirology (Carlton, Vic). 2005;10(2):149-56.

138. Bandeira-Melo C, Singh Y, Cordeiro RS, e Silva PM, Martins MA. Involvement of prostaglandins in the down-regulation of allergic plasma leakage observed in rats undergoing pleural eosinophilia. The British Journal of Pharmacology. 1996;118(8):2192-8.
139. Torres R, Picado C, de Mora F. The PGE2-EP2-mast cell axis: an antiasthma mechanism. Molecular Immunology. 2015;63(1):61-8.
140. Legler DF, Bruckner M, Uetz-von Allmen E, Krause P. Prostaglandin E2 at new glance: novel insights in functional diversity offer therapeutic chances. International Journal of Biochemistry and Cell Biology. 2010;42(2):198-201.
141. Nakanishi M, Rosenberg DW. Multifaceted roles of PGE2 in inflammation and cancer. Seminars in Immunopathology. 2013;35(2):123-37.
142. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. Clinical immunology (Orlando, Fla). 2006;119(3):229-40.
143. Dey I, Lejeune M, Chadee K. Prostaglandin E(2) receptor distribution and function in the gastrointestinal tract. The British Journal of Pharmacology. 2006;149(6):611-23.
144. Krause P, Bruckner M, Uermosi C, Singer E, Groettrup M, Legler DF. Prostaglandin E(2) enhances T-cell proliferation by inducing the costimulatory molecules OX40L, CD70, and 4-1BBL on dendritic cells. Blood. 2009;113(11):2451-60.
145. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. Trends in Immunology. 2002;23(3):144-50.
146. Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, et al. COX-2-derived prostacyclin confers atheroprotection on female mice. Science. 2004;306(5703):1954-7.
147. Legler DF, Krause P, Scandella E, Singer E, Groettrup M. Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors. Journal of Immunology. 2006;176(2):966-73.
148. Regan JW. EP2 and EP4 prostanoid receptor signaling. Life Sciences. 2003;74(2-3):143-53.
149. Schmid A, Thierauch KH, Schleuning WD, Dinter H. Splice Variants of the Human EP3 Receptor for Prostaglandin E2. The European Journal of Biochemistry. 1995;228(1):23-30.
150. Hasegawa H, Negishi M, Ichikawa A. Two isoforms of the prostaglandin E receptor EP3 subtype different in agonist-independent constitutive activity. The Journal of Biological Chemistry. 1996;271(4):1857-60.
151. Duffy SM, Cruse G, Cockerill SL, Brightling CE, Bradding P. Engagement of the EP2 prostanoid receptor closes the K+ channel KCa3.1 in human lung mast cells and attenuates their migration. The European Journal of Immunology. 2008;38(9):2548-56.
152. Feng C, Beller EM, Bagga S, Boyce JA. Human mast cells express multiple EP receptors for prostaglandin E2 that differentially modulate activation responses. Blood. 2006;107(8):3243-50.
153. Gomi K, Zhu FG, Marshall JS. Prostaglandin E2 selectively enhances the IgE-mediated production of IL-6 and granulocyte-macrophage colony-stimulating factor by mast cells through an EP1/EP3-dependent mechanism. The Journal of Immunology (Baltimore, Md : 1950). 2000;165(11):6545-52.
154. Kay LJ, Yeo WW, Peachell PT. Prostaglandin E2 activates EP2 receptors to inhibit human lung mast cell degranulation. The British Journal of Pharmacology. 2006;147(7):707-13.

155. Zaslona Z, Okunishi K, Bourdonnay E, Domingo-Gonzalez R, Moore BB, Lukacs NW, et al. Prostaglandin E(2) suppresses allergic sensitization and lung inflammation by targeting the E prostanoid 2 receptor on T cells. The Journal of Allergy and Clinical Immunology. 2014;133(2):379-87.
156. Claar D, Hartert TV, Peebles RS, Jr. The role of prostaglandins in allergic lung inflammation and asthma. Expert Review of Respiratory Medicine. 2015;9(1):55-72.
157. Main IHM. The inhibitory actions of prostaglandins on respiratory smooth muscle. The British Journal of Pharmacology and Chemotherapy. 1964;22(3):511-9.
158. Picado C, Fernandez-Morata JC, Juan M, Roca-Ferrer J, Fuentes M, Xaubet A, et al. Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. The American Journal of Respiratory and Critical Care Medicine. 1999;160(1):291-6.
159. Pierzchalska M, Szabo Z, Sanak M, Soja J, Szczeklik A. Deficient prostaglandin E2 production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma. The Journal of Allergy and Clinical Immunology. 2003;111(5):1041-8.
160. Szczeklik A. Aspirin-induced asthma: a tribute to John Vane as a source of inspiration. Pharmacological Reports. 2010;62(3):526-9.
161. Grattan CE. Aspirin sensitivity and urticaria. Clinical and Experimental Dermatology. 2003;28(2):123-7.
162. Babu KS, Salvi SS. Aspirin and asthma. Chest. 2000;118(5):1470-6.
163. Morwood K, Gillis D, Smith W, Kette F. Aspirin-sensitive asthma. Internal Medicine Journal. 2005;35(4):240-6.
164. Herrerias A, Torres R, Serra M, Marco A, Roca-Ferrer J, Picado C, et al. Subcutaneous prostaglandin E(2) restrains airway mast cell activity in vivo and reduces lung eosinophilia and Th(2) cytokine overproduction in house dust mite-sensitive mice. International Archives of Allergy and Immunology. 2009;149(4):323-32.
165. Torres R, Perez M, Marco A, Picado C, de Mora F. [A cyclooxygenase-2 selective inhibitor worsens respiratory function and enhances mast cell activity in ovalbumin-sensitized mice]. Archivos de Bronconeumologia. 2009;45(4):162-7.
166. Kunikata T, Yamane H, Segi E, Matsuoka T, Sugimoto Y, Tanaka S, et al. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. Nature Immunology. 2005;6(5):524-31.
167. Farooque SP, Lee TH. Aspirin-sensitive respiratory disease. Annual Review of Physiology. 2009;71:465-87.
168. Peebles RS, Jr., Hashimoto K, Sheller JR, Moore ML, Morrow JD, Ji S, et al. Allergen-induced airway hyperresponsiveness mediated by cyclooxygenase inhibition is not dependent on 5-lipoxygenase or IL-5, but is IL-13 dependent. The Journal of Immunology (Baltimore, Md : 1950). 2005;175(12):8253-9.
169. Wang XS, Wu AY, Leung PS, Lau HY. PGE suppresses excessive anti-IgE induced cysteinyl leucotrienes production in mast cells of patients with aspirin exacerbated respiratory disease. Allergy. 2007;62(6):620-7.
170. Roca-Ferrer J, Perez-Gonzalez M, Garcia-Garcia FJ, Pereda J, Pujols L, Alobid I, et al. Low prostaglandin E2 and cyclooxygenase expression in nasal mucosa fibroblasts of aspirin-intolerant asthmatics. Respirology (Carlton, Vic). 2013;18(4):711-7.

171. Higashi N, Mita H, Ono E, Fukutomi Y, Yamaguchi H, Kajiwara K, et al. Profile of eicosanoid generation in aspirin-intolerant asthma and anaphylaxis assessed by new biomarkers. The Journal of Allergy and Clinical Immunology. 2010;125(5):1084-91.e6.
172. Kowalski ML, Pawliczak R, Wozniak J, Siuda K, Poniatowska M, Iwaszkiewicz J, et al. Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. American Journal of Respiratory and Critical care medicine. 2000;161(2 Pt 1):391-8.
173. Ying S, Meng Q, Scadding G, Parikh A, Corrigan CJ, Lee TH. Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. The Journal of Allergy and Clinical Immunology. 2006;117(2):312-8.
174. Palikhe NS, Sin HJ, Kim SH, Sin HJ, Hwang EK, Ye YM, et al. Genetic variability of prostaglandin E2 receptor subtype EP4 gene in aspirin-intolerant chronic urticaria. The Journal of Human Genetics. 2012;57(8):494-9.
175. Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. American Journal of Respiratory and Critical care medicine. 1999;159(1):31-6.
176. Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E2 on allergen-induced asthma. The Americal Review of Respiratory Disease. 1993;148(1):87-90.
177. Smith AP, Cuthbert MF, Dunlop LS. Effects of inhaled prostaglandins E1, E2, and F2alpha on the airway resistance of healthy and asthmatic man. Clinical Science and Molecular Medicine. 1975;48(5):421-30.
178. Peebles RS, Jr., Hashimoto K, Morrow JD, Dworski R, Collins RD, Hashimoto Y, et al. Selective cyclooxygenase-1 and -2 inhibitors each increase allergic inflammation and airway hyperresponsiveness in mice. American Journal of Respiratory and Critical care medicine. 2002;165(8):1154-60.
179. Selg E, Andersson M, Lastbom L, Ryrfeldt A, Dahlen SE. Two different mechanisms for modulation of bronchoconstriction in guinea-pigs by cyclooxygenase metabolites. Prostaglandins & other lipid mediators. 2009;88(3-4):101-10.
180. Tanaka H, Kanako S, Abe S. Prostaglandin E2 receptor selective agonists E-prostanoid 2 and E-prostanoid 4 may have therapeutic effects on ovalbumin-induced bronchoconstriction. Chest. 2005;128(5):3717-23.
181. Martin JG, Suzuki M, Maghni K, Pantano R, Ramos-Barbon D, Ihaku D, et al. The immunomodulatory actions of prostaglandin E2 on allergic airway responses in the rat. The Journal of Immunology (Baltimore, Md : 1950). 2002;169(7):3963-9.
182. Nishigaki N, Negishi M, Sugimoto Y, Namba T, Narumiya S, Ichikawa A. Characterization of the prostaglandin E receptor expressed on a cultured mast cell line, BNu-2cl3. Biochemical Pharmacology. 1993;46(5):863-9.
183. Chan CL, Jones RL, Lau HY. Characterization of prostanoid receptors mediating inhibition of histamine release from anti-IgE-activated rat peritoneal mast cells. The British journal of Pharmacology. 2000;129(3):589-97.
184. Newcombe DS, Ishikawa Y. The effect of anti-inflammatory agents on human synovial fibroblast prostaglandin synthetase. Prostaglandins. 1976;12(5):849-69.

185. Sahu S, Lynn WS. Metabolism of arachidonic acid in rabbit alveolar macrophages. Inflammation. 1977;2(3):191-7.
186. Loeffler LJ, Lovenberg W, Sjoerdsma A. Effects of dibutyryl-3',5'-cyclic adenosine monophosphage, phosphodiesterase inhibitors and prostaglandin E1 on compound 48-80-induced histamine release from rat peritoneal mast cells in vitro. Biochemical Pharmacology. 1971;20(9):2287-97.
187. Serra-Pages M, Olivera A, Torres R, Picado C, de Mora F, Rivera J. E-prostanoid 2 receptors dampen mast cell degranulation via cAMP/PKA-mediated suppression of IgE-dependent signaling. The Journal of Leukocyte Biology. 2012;92(6):1155-65.
188. Nguyen M, Solle M, Audoly LP, Tilley SL, Stock JL, McNeish JD, et al. Receptors and signaling mechanisms required for prostaglandin E2-mediated regulation of mast cell degranulation and IL-6 production. The Journal of Immunology (Baltimore, Md : 1950). 2002;169(8):4586-93.
189. Kuehn HS, Jung MY, Beaven MA, Metcalfe DD, Gilfillan AM. Distinct PGE2-responder and non-responder phenotypes in human mast cell populations: "all or nothing" enhancement of antigen-dependent mediator release. Immunology letters. 2011;141(1):45-54.
190. Wang XS, Lau HY. Prostaglandin E potentiates the immunologically stimulated histamine release from human peripheral blood-derived mast cells through EP1/EP3 receptors. Allergy. 2006;61(4):503-6.
191. Brown JM, Nemeth K, Kushnir-Sukhov NM, Metcalfe DD, Mezey E. Bone marrow stromal cells inhibit mast cell function via a COX2-dependent mechanism. Clinical and Experimental Allergy. 2011;41(4):526-34.
192. Chung KF. Evaluation of selective prostaglandin E2 (PGE2) receptor agonists as therapeutic agents for the treatment of asthma. Sci STKE. 2005;2005(303):pe47.
193. Kuehn HS, Gilfillan AM. G protein-coupled receptors and the modification of FcepsilonRI-mediated mast cell activation. Immunology Letters. 2007;113(2):59-69.
194. Babina M, Artuc M, Guhl S, Zuberbier T. Retinoic Acid Negatively Impacts Proliferation and MC(TC) Specific Attributes of Human Skin Derived Mast Cells, but Reinforces Allergic Stimulability. International Journal of Molecular Sciences. 2017;18(3):525.
195. Babina M, Guhl S, Artuc M, Trivedi NN, Zuberbier T. Phenotypic variability in human skin mast cells. Experimental Dermatology. 2016;25(6):434-9.
196. Babina M, Guhl S, Artuc M, Zuberbier T. IL-4 and human skin mast cells revisited: reinforcement of a pro-allergic phenotype upon prolonged exposure. Archives of Dermatological Research. 2016;308(9):665-70.
197. Guhl S, Artuc M, Neou A, Babina M, Zuberbier T. Long-term cultured human skin mast cells are suitable for pharmacological studies of anti-allergic drugs due to high responsiveness to FcepsilonRI cross-linking. Bioscience, Biotechnology, and Biochemistry. 2011;75(2):382-4.
198. Guhl S, Neou A, Artuc M, Zuberbier T, Babina M. Skin mast cells develop non-synchronized changes in typical lineage characteristics upon culture. Experimental Dermatology. 2014;23(12):933-5.
199. Mrabet-Dahbi S, Metz M, Dudeck A, Zuberbier T, Maurer M. Murine mast cells secrete a unique profile of cytokines and prostaglandins in response to distinct TLR2 ligands. Experimental Dermatology. 2009;18(5):437-44.

200. Malbec O, Roget K, Schiffer C, Iannascoli B, Dumas AR, Arock M, et al. Peritoneal cell-derived mast cells: an in vitro model of mature serosal-type mouse mast cells. The Journal of Immunology (Baltimore, Md : 1950). 2007;178(10):6465-75.
201. Matsuda H, Kannan Y, Ushio H, Kiso Y, Kanemoto T, Suzuki H, et al. Nerve growth factor induces development of connective tissue-type mast cells in vitro from murine bone marrow cells. The Journal of Experimental Medicine. 1991;174(1):7-14.
202. Nassiri M, Babina M, Dolle S, Edenharter G, Rueff F, Worm M. Ramipril and metoprolol intake aggravate human and murine anaphylaxis: evidence for direct mast cell priming. The Journal of Allergy and Clinical Immunology. 2015;135(2):491-9.
203. Siraganian RP. Automated histamine release. A method for in vitro allergy diagnosis. International Archives of Allergy and Immunology. 1975;49(1-2):108-10.
204. Siraganian RP. Automated histamine analysis for in vitro allergy testing. II. Correlation of skin test results with in vitro whole blood histamine release in 82 patients. The Journal of Allergy and Clinical Immunology. 1977;59(3):214-22.
205. Immunofluorescences: Basic Considerations. In: Radbruch A., Flow Cytometry and Cell Sorting.2000. 38-52 p.
206. Team RDC. R: A language and environment for statistical computing. Vienna, Austria.: R Foundation for Statistical Computing; 2008.
207. Alcorn JF, Ckless K, Brown AL, Guala AS, Kolls JK, Poynter ME, et al. Strain-dependent activation of NF-kappaB in the airway epithelium and its role in allergic airway inflammation. The American Journal of Physiology- Lung Cellular and Molecular Physiology. 2010;298(1):L57-66.
208. Kuroda E, Yamashita U. Mechanisms of enhanced macrophage-mediated prostaglandin E2 production and its suppressive role in Th1 activation in Th2-dominant BALB/c mice. The Journal of Immunology (Baltimore, Md : 1950). 2003;170(2):757-64.
209. Pae S, Cho JY, Dayan S, Miller M, Pemberton AD, Broide DH. Chronic allergen challenge induces bronchial mast cell accumulation in BALB/c but not C57BL/6 mice and is independent of IL-9. Immunogenetics. 2010;62(8):499-506.
210. Serra-Pages M, Torres R, Plaza J, Herrerias A, Costa-Farre C, Marco A, et al. Activation of the Prostaglandin E2 receptor EP2 prevents house dust mite-induced airway hyperresponsiveness and inflammation by restraining mast cells' activity. Clinical and Experimental Allergy. 2015;45(10):1590-600.
211. Yamashita Y, Charles N, Furumoto Y, Odom S, Yamashita T, Gilfillan AM, et al. Cutting edge: genetic variation influences Fc epsilonRI-induced mast cell activation and allergic responses. The Journal of Immunology (Baltimore, Md : 1950). 2007;179(2):740-3.
212. Han H, Thelen TD, Comeau MR, Ziegler SF. Thymic stromal lymphopoietin-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis. The Journal of Clinical Investigation. 2014;124(12):5442-52.
213. Wang M, Takeda K, Shiraishi Y, Okamoto M, Dakhama A, Joetham A, et al. Peanut-induced intestinal allergy is mediated through a mast cell-IgE-FcepsilonRI-IL-13 pathway. The Journal of Allergy and Clinical Immunology. 2010;126(2):306-16, 16.e1-12.

214. Noguchi S, Arakawa T, Fukuda S, Furuno M, Hasegawa A, Hori F, et al. FANTOM5 CAGE profiles of human and mouse samples. Scientific data. 2017;4:170112-.
215. Forrest ARR, Kawaji H, Rehli M, et al. A promoter-level mammalian expression atlas. Nature. 2014;507:462.
216. Machado-Carvalho L, Roca-Ferrer J, Picado C. Prostaglandin E2 receptors in asthma and in chronic rhinosinusitis/nasal polyps with and without aspirin hypersensitivity. Respiratory Research. 2014;15:100.
217. Hizaki H, Segi E, Sugimoto Y, Hirose M, Saji T, Ushikubi F, et al. Abortive expansion of the cumulus and impaired fertility in mice lacking the prostaglandin E receptor subtype EP(2). Proceedings of the National Academy of Sciences of the United states of America. 1999;96(18):10501-6.
218. Norel X, de Montpreville V, Brink C. Vasoconstriction induced by activation of EP1 and EP3 receptors in human lung: effects of ONO-AE-248, ONO-DI-004, ONO-8711 or ONO-8713. Prostaglandins and Other Lipid Mediators. 2004;74(1-4):101-12.
219. Mori A, Ishii T, Kuroki T, Shigeta N, Sakamoto K, Nakahara T, et al. The prostanoid EP(2) receptor agonist ONO-AE1-259-01 protects against glutamate-induced neurotoxicity in rat retina. The European Journal of Pharmacology. 2009;616(1-3):64-7.
220. Berghea EC, Popa LO, Dutescu MI, Meirosu M, Farcasanu IC, Berghea F, et al. Association of Leukotriene C4 Synthase A-444C Polymorphism with Asthma and Asthma Phenotypes in Romanian Population. Maedica. 2015;10(2):91-6.
221. Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, et al. Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion. Nature medicine. 2009;15(6):633-40.
222. Kabashima K, Sakata D, Nagamachi M, Miyachi Y, Inaba K, Narumiya S. Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. Nature medicine. 2003;9(6):744-9.
223. Greenhough A, Smartt HJ, Moore AE, Roberts HR, Williams AC, Paraskeva C, et al. The COX-2/PGE2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis. 2009;30(3):377-86.
224. Wang MT, Honn KV, Nie D. Cyclooxygenases, prostanoids, and tumor progression. Cancer metastasis reviews. 2007;26(3-4):525-34.
225. Melillo E, Woolley KL, Manning PJ, Watson RM, O'Byrne PM. Effect of inhaled PGE2 on exercise-induced bronchoconstriction in asthmatic subjects. American Journal of Respiratory and Critical care medicine. 1994;149(5):1138-41.
226. Sestini P, Armetti L, Gambaro G, Pieroni MG, Refini RM, Sala A, et al. Inhaled PGE2 prevents aspirin-induced bronchoconstriction and urinary LTE4 excretion in aspirin-sensitive asthma. American Journal of Respiratory and Critical care medicine. 1996;153(2):572-5.
227. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. The Journal of Allergy and Clinical Immunology. 2015;135(3):676-81 e1.
228. Phipps RP, Stein SH, Roper RL. A new view of prostaglandin E regulation of the immune response. Immunology Today. 1991;12(10):349-52.

229. Sastre B, del Pozo V. Role of PGE2 in asthma and nonasthmatic eosinophilic bronchitis. Mediators of Inflammation. 2012;2012:645383.
230. Chambers LS, Black JL, Ge Q, Carlin SM, Au WW, Poniris M, et al. PAR-2 activation, PGE2, and COX-2 in human asthmatic and nonasthmatic airway smooth muscle cells. American Journal of Physiology- Lung Cellular and Molecular Physiology. 2003;285(3):L619-27.
231. Wittenberg M, Nassiri M, Francuzik W, Lehmann K, Babina M, Worm M. Serum levels of 9alpha,11beta-PGF2 and apolipoprotein A1 achieve high predictive power as biomarkers of anaphylaxis. Allergy. 2017;72(11):1801-5.
232. Akasaka H, Ruan KH. Identification of the two-phase mechanism of arachidonic acid regulating inflammatory prostaglandin E2 biosynthesis by targeting COX-2 and mPGES-1. Archives of biochemistry and biophysics. 2016;603:29-37.
233. Mehdawi LM, Satapathy SR, Gustafsson A, Lundholm K, Alvarado-Kristensson M, Sjolander A. A potential anti-tumor effect of leukotriene C4 through the induction of 15-hydroxyprostaglandin dehydrogenase expression in colon cancer cells. Oncotarget. 2017;8(21):35033-47.
234. Tai HH, Ensor CM, Tong M, Zhou H, Yan F. Prostaglandin catabolizing enzymes. Prostaglandins & other lipid mediators. 2002;68-69:483-93.
235. Zhang Y, Desai A, Yang SY, Bae KB, Antczak MI, Fink SP, et al. Tissue Regeneration. Inhibition of the prostaglandin-degrading enzyme 15-PGDH potentiates tissue regeneration. Science. 2015;348(6240):2340.
236. Hartney JM, Coggins KG, Tilley SL, Jania LA, Lovgren AK, Audoly LP, et al. Prostaglandin E2 protects lower airways against bronchoconstriction. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2006;290(1):L105-L13.
237. Liu T, Laidlaw TM, Katz HR, Boyce JA. Prostaglandin E₂ deficiency causes a phenotype of aspirin sensitivity that depends on platelets and cysteinyl leukotrienes. Proceedings of the National Academy of Sciences of the United states of America. 2013;110(42):16987-92.
238. Kowalski ML, Borowiec M, Kurowski M, Pawliczak R. Alternative splicing of cyclooxygenase-1 gene: altered expression in leucocytes from patients with bronchial asthma and association with aspirin-induced 15-HETE release. Allergy. 2007;62(6):628-34.
239. Flower R. What are all the things that aspirin does? British Medical Journal. 2003;327(7415):572-3.
240. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proceedings of the National Academy of Sciences of the United States of America. 1993;90(24):11693-7.
241. Picado C. Mechanisms of aspirin sensitivity. Current Allergy and Asthma Reports. 2006;6(3):198-202.
242. Laidlaw TM, Cahill KN. Current Knowledge and Management of Hypersensitivity to Aspirin and NSAIDs. The Journal of Allergy and Clinical Immunology: In Practice. 2017;5(3):537-45.
243. Serra M, Torres R, Herrerias A, De Mora F. Early protection of Prostaglandin E2 receptor 2 agonist against house dust mite induced pathology in mice's airway. Allergy. 2009;64:540-.

244. Herrerias A, Torres R, Serra M, Marco A, Pujols L, Picado C, et al. Activity of the cyclooxygenase 2-prostaglandin-E prostanoid receptor pathway in mice exposed to house dust mite aeroallergens, and impact of exogenous prostaglandin E2. The Journal of Inflammation (London, England). 2009;6:30.
245. Hattori R, Shimizu S, Majima Y, Shimizu T. Prostaglandin E2 receptor EP2, EP3, and EP4 agonists inhibit antigen-induced mucus hypersecretion in the nasal epithelium of sensitized rats. The Annals of Otology, Rhinology, and Laryngology. 2009;118(7):536-41.
246. Ueta M, Matsuoka T, Narumiya S, Kinoshita S. Prostaglandin E receptor subtype EP3 in conjunctival epithelium regulates late-phase reaction of experimental allergic conjunctivitis. The Journal of Allergy and Clinical Immunology. 2009;123(2):466-71.
247. Hattori H, Kato M, Tamanaka M, Aoki T, Furuhama K, Manabe S. Development of a Novel Mouse Anaphylaxis Model Produced by Intermittent Intravenous Injections of Ovalbumin without Adjuvant. Journal of Toxicologic Pathology. 2007;20(4):237-44.
248. Pablos-Tanarro A, Lopez-Exposito I, Lozano-Ojalvo D, Lopez-Fandino R, Molina E. Antibody Production, Anaphylactic Signs, and T-Cell Responses Induced by Oral Sensitization With Ovalbumin in BALB/c and C3H/HeOuJ Mice. Allergy, asthma & immunology research. 2016;8(3):239-45.
249. Marco-Martin G, La Rotta Hernandez A, Vazquez de la Torre M, Higaki Y, Zubeldia JM, Baeza ML. Differences in the Anaphylactic Response between C3H/HeOuJ and BALB/c Mice. International archives of Allergy and Immunology. 2017;173(4):204-12.
250. Orgel K, Smeekens JM, Ye P, Fotsch L, Guo R, Miller DR, et al. Genetic diversity between mouse strains allows identification of the CC027/GeniUnc strain as an orally reactive model of peanut allergy. The Journal of Allergy and Clinical Immunology. 2019;143(3):1027-37.e7.
251. Kelada SN, Wilson MS, Tavarez U, Kubalanza K, Borate B, Whitehead GS, et al. Strain-dependent genomic factors affect allergen-induced airway hyperresponsiveness in mice. American journal of Respiratory Cell and Molecular Biology. 2011;45(4):817-24.
252. Backlund MG, Mann JR, Holla VR, Buchanan FG, Tai HH, Musiek ES, et al. 15-Hydroxyprostaglandin dehydrogenase is down-regulated in colorectal cancer. The Journal of Biological Chemistry. 2005;280(5):3217-23.
253. Tseng-Rogenski S, Gee J, Ignatoski KW, Kunju LP, Bucheit A, Kintner HJ, et al. Loss of 15-hydroxyprostaglandin dehydrogenase expression contributes to bladder cancer progression. The American Journal of Pathology. 2010;176(3):1462-8.
254. Eruslanov E, Kaliberov S, Daurkin I, Kaliberova L, Buchsbaum D, Vieweg J, et al. Altered expression of 15-hydroxyprostaglandin dehydrogenase in tumor-infiltrated CD11b myeloid cells: a mechanism for immune evasion in cancer. The Journal of Immunology (Baltimore, Md : 1950). 2009;182(12):7548-57.
255. Yan M, Myung SJ, Fink SP, Lawrence E, Lutterbaugh J, Yang P, et al. 15-Hydroxyprostaglandin dehydrogenase inactivation as a mechanism of resistance to celecoxib chemoprevention of colon tumors. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(23):9409-13.
256. Pereira C, Queiros S, Galaghar A, Sousa H, Pimentel-Nunes P, Brandao C, et al. Genetic variability in key genes in prostaglandin E2 pathway (COX-2, HPGD, ABCC4 and SLCO2A1) and their involvement in colorectal cancer development. PloS one. 2014;9(4):e92000.

257. Kalinski P. Regulation of Immune Responses by Prostaglandin E(2). The Journal of Immunology (Baltimore, Md : 1950). 2012;188(1):21-8.
258. Kawahara K, Hohjoh H, Inazumi T, Tsuchiya S, Sugimoto Y. Prostaglandin E2-induced inflammation: Relevance of prostaglandin E receptors. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. 2015;1851(4):414-21.
259. St-Jacques B, Ma W. Preferred recycling pathway by internalized PGE2 EP4 receptor following agonist stimulation in cultured dorsal root ganglion neurons contributes to enhanced EP4 receptor sensitivity. Neuroscience. 2016;326:56-68.
260. Kay LJ, Gilbert M, Pullen N, Skerratt S, Farrington J, Seward EP, et al. Characterization of the EP receptor subtype that mediates the inhibitory effects of prostaglandin E2 on IgE-dependent secretion from human lung mast cells. Clinical and Experimental Allergy. 2013;43(7):741-51.
261. Morimoto K, Shirata N, Taketomi Y, Tsuchiya S, Segi-Nishida E, Inazumi T, et al. Prostaglandin E₂–EP3 Signaling Induces Inflammatory Swelling by Mast Cell Activation. The Journal of Immunology. 2014;192(3):1130-7.
262. Pantazaka E, Taylor EJ, Bernard WG, Taylor CW. Ca(2+) signals evoked by histamine H1 receptors are attenuated by activation of prostaglandin EP2 and EP4 receptors in human aortic smooth muscle cells. The British Journal of Pharmacology. 2013;169(7):1624-34.
263. Srivastava K, Sampson HA, Charles W. Emala S, Li X-M. The anti-asthma herbal medicine ASHMI acutely inhibits airway smooth muscle contraction via prostaglandin E2 activation of EP2/EP4 receptors. American Journal of Physiology-Lung Cellular and Molecular Physiology. 2013;305(12):L1002-L10.
264. Torres-Atencio I, Ainsua-Enrich E, de Mora F, Picado C, Martin M. Prostaglandin E2 prevents hyperosmolar-induced human mast cell activation through prostanoid receptors EP2 and EP4. PloS one. 2014;9(10):e110870.
265. Nataraj C, Thomas DW, Tilley SL, Nguyen M, Mannon R, Koller BH, et al. Receptors for prostaglandin E-2 that regulate cellular immune responses in the mouse. The Journal of Clinical Investigation. 2001;108(8):1229-35.
266. Ratcliffe MJ, Walding A, Shelton PA, Flaherty A, Dougall IG. Activation of E-prostanoid₄ and E-prostanoid₂ receptors inhibits TNF-α release from human alveolar macrophages. The European Respiratory Journal. 2007;29(5):986-94.
267. Wen R, Jou S-T, Chen Y, Hoffmeyer A, Wang D. Phospholipase Cγ2 Is Essential for Specific Functions of FcεR and FcγR. The Journal of Immunology. 2002;169(12):6743-52.
268. Gilfillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. Nature Reviews Immunology. 2006;6(3):218-30.
269. Pereira PAT, Assis PA, Prado MKB, Ramos SG, Aronoff DM, de Paula-Silva FWG, et al. Prostaglandins D(2) and E(2) have opposite effects on alveolar macrophages infected with Histoplasma capsulatum. The Journal of Lipid Research. 2018;59(2):195-206.
270. Hirasawa N, Santini F, Beaven MA. Activation of the mitogen-activated protein kinase/cytosolic phospholipase A2 pathway in a rat mast cell line. Indications of different pathways for release of arachidonic acid and secretory granules. The Journal of Immunology (Baltimore, Md : 1950). 1995;154(10):5391-402.

271. Roa M, Paumet F, Le Mao J, David B, Blank U. Involvement of the ras-like GTPase rab3d in RBL-2H3 mast cell exocytosis following stimulation via high affinity IgE receptors (Fc epsilonRI). The Journal of Immunology. 1997;159(6):2815-23.
272. Zhu W, Downey JS, Gu J, Di Padova F, Gram H, Han J. Regulation of TNF expression by multiple mitogen-activated protein kinase pathways. The Journal of Immunology (Baltimore, Md : 1950). 2000;164(12):6349-58.
273. Scherle PA, Jones EA, Favata MF, Daulerio AJ, Covington MB, Nurnberg SA, et al. Inhibition of MAP kinase kinase prevents cytokine and prostaglandin E2 production in lipopolysaccharide-stimulated monocytes. The Journal of Immunology (Baltimore, Md : 1950). 1998;161(10):5681-6.
274. Minami M, Shimizu K, Okamoto Y, Folco E, Ilasaca ML, Feinberg MW, et al. Prostaglandin E receptor type 4-associated protein interacts directly with NF-kappaB1 and attenuates macrophage activation. The Journal of Biological Chemistry. 2008;283(15):9692-703.
275. Paniagua-Herranz L, Gil-Redondo JC, Queipo MJ, Gonzalez-Ramos S, Bosca L, Perez-Sen R, et al. Prostaglandin E2 Impairs P2Y2/P2Y4 Receptor Signaling in Cerebellar Astrocytes via EP3 Receptors. Frontiers in Pharmacology. 2017;8:937.
276. Kuehn HS, Beaven MA, Ma H-T, Kim M-S, Metcalfe DD, Gilfillan AM. Synergistic activation of phospholipases Cgamma and Cbeta: a novel mechanism for PI3K-independent enhancement of FcepsilonRI-induced mast cell mediator release. Cellular signalling. 2008;20(4):625-36.
277. Luschnig-Schratl P, Sturm EM, Konya V, Philipose S, Marsche G, Fröhlich E, et al. EP4 receptor stimulation down-regulates human eosinophil function. Cellular and Molecular Life Sciences. 2011;68(21):3573-87.
278. Birrell MA, Maher SA, Dekkak B, Jones V, Wong S, Brook P, et al. Anti-inflammatory effects of PGE₂ in the lung: role of the EP₄ receptor subtype. Thorax. 2015;70(8):740-7.
279. Buckley J, Birrell MA, Maher SA, Nials AT, Clarke DL, Belvisi MG. EP4 receptor as a new target for bronchodilator therapy. Thorax. 2011;66(12):1029-35.

ACKNOWLEDGEMENT

I would like to thank my supervisor Prof. Dr. Margitta Worm for offering me the opportunity to be a part of her research group, and work towards my PhD degree. I am grateful for her constant guidance and encouragement especially during difficult times in the PhD pursuit. I appreciate her contribution in terms of time, ideas, and funding to make my PhD experience productive. I would like to thank Dr. Magda Babina for all her contributions in terms of time, inspiring ideas and constant guidance which shaped up my work. I am thankful to her for all the discussions we had which immensely increased my knowledge of the project. I am also thankful to all my doctoral committee members for their excellent review of my thesis and their valuable feedback.

I would like to thank Dr. Maria Nassiri, who guided me during the initial years of my PhD. I would like to thank the current and past members of my research group for providing a healthy environment for work and discussions especially Dr. Vandana Kumari and Dr. Diana Willmes for their support and valuable friendship. I am especially grateful to Dennis Ernst for his constant help with everything in the lab.

I would like to express my immense gratitude to my parents (Shri Rakesh Rastogi & Smt. Madhu Rastogi) for always encouraging me to pursue my dreams, for their unconditional support and for setting an outstanding example for my life. I could not have done this without their belief in my abilities. I am truly grateful to my siblings (Dr. Shreya Rastogi and Yash Rastogi) for their incredible support, encouragement and unbending belief in me. I am immensely grateful to my friend and now husband (Anil Kumar), for being immensely patient and offering complete support during my PhD. He truly believed in my potential and constantly encouraged me to look at problems objectively and not give up. His patience and support especially during the final stages of my PhD are greatly appreciated.

I recognize that this research would not have been possible without the financial support from DFG.

SELBSTÄNDIGKEITSERKLÄRUNG / DECLARATION

Hiermit versichere ich, Shruti Rastogi, die vorliegende Dissertation selbständig erarbeitet und verfasst zu haben. Es wurden keine weiteren Quellen und Hilfsmittel als die hier angegebenen verwendet.

I hereby declare that I, Shruti Rastogi, have worked and written this dissertation independently and did not use other than the listed support. This thesis does neither exists in the same or similar form nor submitted to another examination procedure.